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LACK OF EFFECTS OF HIGH LEVEL CARBOXYHEMOGLOBIN ON COMPENSATORY TRACKING AND EEG: PROTOCOL 3

FINAL REPORT

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The effects of 16-23% carboxyhemoglobin (COHb) were studied on compensatory tracking behavior in healthy young men. The COHb was produced by exposure to a bolus of high concentration of carbon monoxide (CO), followed by exposure to a continuous low level concentration of CO (223 ppm) designed to maintain the COHb value throughout the experiment (approximately 130 minutes). The

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compensatory tracking task consisted of trying to maintain a constantly moving spot in the center of an oscilloscope screen. The electroencephalogram (EEG) was recorded during exposure.

No significant effect of the COHb was found on the tracking task or EEG power spectrum. Even after extensive post hoc exploratory analyses, no suggestion of a plausible effect on tracking or EEG was found. The planned significance test had a moderate to high statistical power against effects of interesting size.

The finding of no effect was somewhat surprising since other research had demonstrated effects of lower COHb levels on similar tasks. The level of COHb used in this study is considered to be extremely high. The differences in findings may reflect (a) inadequate statistical power for the size of effect (b) differences in task and variables between studies (c) differences due to COHb formation rates between studies or (d) peculiarities in the dose effects function of COHb.

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EXECUTIVE SUMMARY

One of the most reliable behavioral effects of human exposure to carbon monoxide (CO) is the detrimental effect on the accuracy of compensatory tracking. Effects on tracking have been reported at carboxyhemoglobin (COHb) levels of 5% (Putz et al., 1976, 1979) and at 8% (Benignus et al., 1987a). The design of the task variables in previous studies made understanding of the effect more difficult. In an effort to further explore the reliability of this effect as reported in the literature, the present experiment was designed and executed. It was further of interest to explore the effects of rapid COHb formation since environmental situations occur in which rapid formation would be expected and also experiments could be conducted more quickly.

Nineteen healthy young men were given a compensatory tracking task. This task consisted of trying to keep a spot of light in the center of an oscillosope screen while a computer generated signal attempted to move the spot above or below center. The subjects could counteract the spot motion by pushing or pulling on a joystick. The forcing function (FF), a signal which drove the spot away from center, was mathematically constructed to simplify interpretation of Fourier decomposition of the performance. Earlier studies used FFs which were (a) more predictable and (b) mathematically more difficult to interpret. The subjects performed the task for 11 work periods of 2.67 minutes length, with 10 minute rests interspersed.

The target value of COHb was selected to be 19%. So large a value was selected in order to produce large, frank effects.

Persons who have COHb levels as high as this are frequently reported to have mild headaches, dizziness and nausea and are usually given oxygen therapy in cases of accidental CO exposures (Friedman, 1980; Klingberg, 1983; Klaassen, 1985; Goldfrank et al., 1986). This was considered the largest CO exposure which could be safely given to healthy young men.

Subjects in the CO group breathed a CO/air mixture from a Douglas bag for approximately 2.67 minutes and then breathed room air with a low level (223 ppm) CO to maintain the COHb level for the duration of the experiment (approximately 130 minutes). The COHb levels at the end of the experiment ranged from 16.4 to 22.8%, with a mean of 18.3% in the CO exposed group. Levels ranged from .6 to 1.6% with a mean of 1.0% in the control group.

No significant effect of COHb on tracking was found by a statistical test which had moderate to high power against alternatives of interesting size. Extensive post hoc exploratory analyses failed to find any suggestion of a plausible effect of COHb on tracking or any of the derived spectrum measures of tracking. No indication of an effect of COHb on EEG spectra or in the number of symptoms reported between the two groups was found.

Several task and procedural parameters differed in the present study with respect to earlier work. The FF had a different mathematical structure thereby making tracking more difficult. The present study did not include the background task of light-flash monitoring as in past work. The work/rest schedule was different from that used in previous studies. These differences could have decreased the sensitivity of the

experiment. For so high a level of COHb, however, it would be surprising if these kinds of changes could obviate an effect (if an effect, in fact, exists in the population).

Another difference between the present and previous studies was the rate of COHb formation. The present study used rapid formation while previous work used slow formation. It is possible that this variable could account for the differences in results between studies. If, however, the important variable in CO effects is COHb level, the rate of COHb formation should not matter.

It is possible that "compensatory" changes in brain blood supply are more sensitive to rapid COHb changes than to slow ones. If so, it may be conjectured that compensation in the rapid buildup would be more effective, thus preventing effects.

In previous studies, using slow COHb formation, 5% COHb produced larger effects (Putz _et al._) than did 8% (Benignus _et al._). In this study using rapid COHb formation, 18% COHb produced no demonstrable effect. If rate of COHb formation is not important, then it is possible that the dose effects function relating COHb to tracking and EEG is not monotonic. A mechanism for the possible non-monotonic COHb dose effects function might lie in the sensitivity of the "compensatory" blood supply to the brain to the absolute level of COHb.

Regardless of the reason for the lack of significant effects of high level COHb on tracking and EEG spectra, the finding is somewhat surprising and potentially important. It would be theoretically and practically important to know that the effects

of COHb were sensitive to certain task parameters. Much information would be gained about the mechanism of COHb effects if the effects were shown to be mitigated by rate of COHb formation. It would be a matter of both regulatory and theoretical concern if the dose effect function of COHb were not monotonic. Any, or a combination of the above speculations may be true.

FORWARD

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TABLE OF ABBREVIATIONS

CO	Carbon monoxide, an invisible, odorless gas which is the byproduct of incomplete combustion.
COHb	Carboxyhemoglobin, a measure of the level of carbon monoxide in the blood.
EEG	Electroencephalogram, the recording of small electrical signals generated by the brain; the so-called brain wave.
FF	Forcing function, a signal or voltage which drives the oscilloscope spot away from the target.
Hz	Hertz, a measure of frequency of an oscillating wave in cycles per second.
p	Probability of an event or outcome of a test of significance.
ppm	Parts per million.

INTRODUCTION

One of the most reliable behavioral effects of human exposure to carbon monoxide (CO) is the detrimental effect on accuracy of compensatory tracking. Putz *et al.* (1976, 1979) reported that carboxyhemoglobin (COHb) levels of 5%, formed over a period of four hours, reduced tracking accuracy. The two studies of Putz *et al.* were especially credible since (a) appropriate and conservative statistical methods were used (b) means were ordinal in time, and therefore in COHb and (c) the two studies used different experimental designs and still achieved the same results.

In an effort to further explore the reliability of the finding, Benignus *et al.* (1987a) utilized the same task and experimental design (insofar as was possible) as Putz *et al.* (1976). The COHb level at the end of the fourth hour in the latter study was near 8%. The direction of the effect reported in Benignus *et al.* was the same as reported by Putz *et al.*, but the size of the effect was smaller. In addition, the variability of the data in the latter study was larger. In the Putz *et al.* study the mean error score of the CO group was about .33 units greater than the control group's at the end of the fourth hour (as corrected for the initial differences between groups). In the Benignus *et al.* study, however, the CO group's error score was only approximately .13 units greater (similarly corrected) than the control group's. The latter was somewhat surprising since the COHb was larger for the smaller effect. In view of the greater variance of the data in the study by Benignus *et al.*, it cannot be stated with certainty that the mean effect was

accurately measured. The differences and the analysis of the data are fully explained in Benignus *et al.* (1987a).

In the research leading to the publication by Benignus *et al.* (1983), the electroencephalogram (EEG) was collected and its spectrum analyzed. Exploratory analyses were performed to determine whether the EEG spectra were affected by CO exposure. Results indicated that, although the tests were not significant, the amplitude of EEG alpha activity might be reduced by CO exposure. The results were sufficiently promising to justify further exploration.

The present study was designed and executed in an effort to obtain clear, frank effects. High levels of COHb were used as well as a new FF which was constructed to facilitate Fourier analysis of the performance.

METHODS

Subjects

Subjects consisted of 19 men, 9 exposed to CO and 10 controls. The mean age was 24.3 years, with a standard deviation of 3.9 and a range of 18.8 - 32.2 years. Subjects were recruited from the community of the University of North Carolina and the surrounding areas by advertisement. Potential subjects were screened before participation by being given a general health questionnaire (Duke Medical History form) and a personality survey (Minnesota Multiphasic Personality Inventory). All persons who were normal within statistical limits were then given physical examinations by a physician with special emphasis on cardiac health. The latter included a twelve lead

electrocardiogram (ECG). Persons showing no signs of abnormality were then given an exercise ECG (Bruce Protocol) in an effort to assure that no occult cardiac abnormalities were present. All subjects were paid for their participation in the experiment and were informed of the findings.

Compensatory Tracking Task

The subject's task was to control the position of a spot of light on the screen of an oscilloscope by pushing or pulling on a joystick manipulandum. The spot was continually driven away from the center of the screen by a computer generated forcing function. As the spot was driven away from center, the subject was required to apply compensatory pressure to the joystick and keep the spot centered. Pushing (forward) on the joystick tended to move the spot upward on the screen.

In previous studies (Putz *et al.* 1976, 1979; Benignus *et al.* 1987a) the subjects had performed two simultaneous tasks: a tracking task, as described above, and a light monitoring task which required them to press a button each time a light flash in a series of flashes was slightly brighter than others. The performance on the monitoring task was only slightly affected by the CO exposure in the studies by Putz *et al.* and not at all in the study by Benignus *et al.* The only reason for including the monitoring task in the present experiment would have been to produce extra difficulty. Since the FF could be manipulated to increase task difficulty, it was decided to exclude the light monitoring task in the present study.

Table 1 gives the schedule of events during the day of the experiment. The events in the present study are described

graphically in Figures 1 and 2. Figure 1 is a schedule of events over the entire experimental session, showing the timing of work and rest periods. Figure 2 is a detailed graph of the events within one of the work periods shown in Figure 1. These figures and the table may be consulted in the following detailed description of the protocol.

For the duration of the experiment the subject performed the tracking task on an alternating work/rest cycle. Tracking was performed for 2.67 minutes followed by a rest of 10 minutes. There were 11 work/rest cycles so that the experiment lasted approximately 130 minutes (the last rest period was not completed). No effort was made to use a work/rest cycle similar to previous studies. If the effect of the CO exposure were upon the perceptual/motor aspect of behavior, as all evidence suggested, the exact schedule would not be critical.

Subjects were trained for approximately one hour before the experiment began. Training continued until an accuracy criterion was achieved, even if more than an hour was required. If, however, the subject could not reach criterion in 90 minutes of training, that subject was not used. Distributed practice with frequent feedback was given. At the start of the training session, the spot movement was slowed to help the subject learn the task. The rate of spot motion was gradually increased until the subject had acnieved the required performance.

Forcing Functions (FFs). It was desired to generate FFs which would have simpler Fourier transforms than the FF used by Putz *et al.* Licklider (1960) recommended using Fourier analysis

of tracking performance. A convenient FF for this method is a time function having a flat power spectrum between the lower and upper frequency limits. The frequency limits were to be selected to test the subject's tracking ability at a wide range of spot movement speeds.

From pilot studies and the work of others (Notterman and Weitzman, 1981; Bosser, 1984) it was decided to construct forcing functions which contained frequencies between .15 and .6 Hz. The decision was made on the basis of the ability of human subjects to accurately track spots moving at those speeds but not much faster. The task was so constructed as to require 11 FFs of 2.67 minute duration (see above) each different from the others. Within each FF the spot movement was to be unpredictable.

Ten spectrally identical 30-second FF segments were constructed using the method of Fourier synthesis (Bendat and Piersol, 1966). For each FF segment the following steps were followed: (a) equal amplitudes were specified for a spectrum for the desired frequency bands (b) the phase of each frequency band was selected randomly and (c) the resulting spectrum was inverse Fourier transformed to produce a time series of the desired characteristics. Since each of the resulting time series had a randomly selected phase angle for each frequency band, the resulting time function was random and therefore different from the other members of its family. To assure that the FF segments all began and ended at a zero crossing and therefore would produce spot motion which would smoothly depart from center screen at the beginning and end of each FF segment, individual segments were selected for this characteristic. Figure 3 shows

the 10 FF segments which were generated, plotted as functions of time.

Due to constraints of processing speed and available memory in the control and data acquisition system (CDAS) used to control the experiment, the FFs were constructed off-line. Each 2.67 minute FF was constructed by catenation of five 30 second FF segments (described above). Approximately 2.5 seconds were required between each 30 second segment of FF in order to read a new segment and to store data from the experiment. During this 2.5 second period, the spot remained motionless. For each 2.67 minute work period, a different order of FF segments was used so that each resulting 2.67 minute FF was unique. By use of the above 10 FF segments it was possible to construct unique orders of FF segments for each work period. Table 2 shows the order of the FF segments for each of the eleven 2.67 minute work periods in the experiment. Work periods 1-10 used unique FFs, but work period 11 was a repetition of work period 1. This did not matter since data from work period 11 were to be disregarded (see Statistics). In this way none of the FFs were predictable but each subject had the same FF as the others.

Previous studies used half sinusoid waves as FFs. The random FFs of the present study were much less predictable. They were also more difficult to track due to the higher frequency components they contained. According to the results of Putz *et al.*, increased task difficulty would make the task more sensitive to disruption by COHb.

Apparatus. The oscilloscope used to present the moving spot was a Tektronics model 5110 with a P31 phosphor cathode ray tube without graticule. A stationary spot of light was placed in the center of the screen as a center reference marker by use of one of the channels. The FF was fed into the vertical amplifier of another channel via a digital to analog converter from the CDAS. The subject was seated in front of a small table on which the oscilloscope was placed. The Joystick (Measurement Systems model PN 436) was mounted on a platform extending from the table. The center of the ball top of the joystick was located 30.5 cm (12 inches) below the center and 18 cm (7 inches) in front of the center of the oscilloscope screen. A pressure of 2.4 kg (5.3 lbs) was required to deflect the spot from the center of the screen to the top. The joystick was a pressure transducer which did not move to the application of pressure.

The tracking apparatus and table were contained inside an acoustically isolated, double walled audiometric booth (Tracor RE-242C) with inside dimensions of approximately 2x2x2 m. (approximately 6x6x6 feet). The booth had been modified by the installation of a negative pressure, humidity and temperature controlled air conditioning system. The inside temperature was 24 degrees C, and the relative humidity was 45%. Lighting was provided by a 60 watt incandescent bulb in a "bullet" fixture, pointed at the beige interior ceiling of the chamber.

The CDAS was an IBM XT microcomputer with 640K of memory, two hard disks and one floppy disk drive. Hardware interfaces consisted of Tecmar boards and other specially constructed hardware. Programming was done in Microsoft Fortran. The FF

segments were stored on hard disk and were sent to the 12-bit digital to analog converter at a rate of 100 samples per second.

The joystick pressure was sampled by an analog to digital converter at 100 samples per second, but only every fifth sample was kept for later analysis. The reduced, digitized time function representing the joystick pressure response was stored on hard disk for later analysis. An on-line computation of the mean absolute deviation (MAD) score was also displayed and printed for each 30 second segment of FF.

Electroencephalogram (EEG)

The EEG was collected from the left occipital site (international 10-20 system), measured with respect to linked mastoids. EEG signals were amplified by a Beckman Dynograph system with filters set to produce a band pass between 1 and 30 Hz (3dB points). Amplified EEG signals were analog to digital converted at a rate of 125 samples per second, using background programs, while the tracking task was in progress. The digitized signals were stored on hard disk for later analysis. The electro-oculogram was simultaneously collected and stored for use in the rejection of low frequency eye movement contaminated EEG.

The EEG was collected during the middle three 30 second segments of tracking within each 2.67 minute workperiod. Thus EEG collection began 30 seconds after tracking began. At the end of each of the middle three 30 second segments the EEG data were written to hard disk. Thus for each of the eleven 2.67 minute work periods, there were three 30 second segments of EEG.

CO Exposure.

Transient Exposure. To produce a rapid rise in COHb, the subject breathed from a Douglas bag (Hans Rudolph, 100 liter model 6100) which contained a mixture of breathing air and CO. The concentration in the bag was calculated by the regression equation of Stewart *et al.* (1973) based upon the subject's empirical minute ventilation. Minute ventilation was measured in a bag breathing situation during practice and training. The volume of the gas in the bag was calculated so that the subject would empty the bag in approximately 2.67 minutes.

The subject breathed through a mouthpiece and one-way valve assembly (Hans Rudolph model 1400 with a Vacumed model 1002 rubber mouthpiece). The mouthpiece assembly was mounted in front of the oscilloscope screen so as to place the subject's eyes at the vertical and horizontal center and 30.5 cm (12 inches) from the screen. At all times when the subject was engaged in tracking, he breathed through the mouthpiece. Before and after bag breathing, the air supply was room air (with CO added for CO exposed subjects). At the onset of bag breathing, a Hans Rudolph model 8500 pneumatic valve was switched from room air to the bag as the air source. When the subject had evacuated the bag a vacuum sensor was tripped and the pneumatic valve returned to the room air position. The subject continued to track during the bag breathing period. If the subject was a control subject, the bag contained only breathing grade air. Otherwise the bag contained an air-CO mixture calculated as above.

Maintenance CO Level. While the subject was bag breathing, if the subject was being exposed to CO, a low level of CO was

introduced into the room air (223 ppm target value) which was intended to maintain the level of COHb built up during bolus exposure. Chamber levels were maintaned with a closed-loop controller to an accuracy of approximately 5 ppm. When the subject resumed breathing room air via the mouthpiece, the room air CO level had stabilized to the desired level.

Double Blind Procedures. In all cases neither the subject nor anyone who had contact with the subject was informed of the exposure condition. This "double blind" procedure was achieved by having a gas controller who had no other duties in the experiment than to (a) mix the gasses for the bag and (b) visually monitor the gas in the chamber air via the gas meter and the stripchart recording of the gas level.

After the experiment was begun, no further social contact with the subject occurred. Even though the subject was carefully monitored, no one spoke to him, and *vice versa*, for his remaining time in the experimental chamber. If the subject did initiate contact by speaking to the experimenters via the intercom, one of two actions was taken. If the communication was declaratory, no response was made. If the communication was interrogatory, a reply was made, the experiment was terminated, and the subject's data were discarded. Thus, the experimenter's blind was able to be broken immediately after the beginning of the experiment.

Blood Sampling and COHb Measurement. Two three-ml vacutainers of venous blood were drawn before and after the experiment from each subject. COHb was measured in triplicate immediately after blood was drawn by use of an Instrumentation

Laboratories model 282 CO oximeter. Triplicate values were averaged to produce a final pre and post COHb value.

Pilot Studies of Exposure Method.

Appendix 1 is a report of the pilot studies which (a) tested the exposure methods (b) documented the safety of the method and (c) performed *post hoc* analyses of the COHb levels in the main study. The methods and results of the pilot and *post hoc* studies are given in Appendix 1.

Statistics.

Confirmatory Analysis. In order to maximize statistical power for a fixed sample size and type I error rate, a single *a priori* hypothesis was chosen. To avoid effects of subject anticipation of the end of the experiment (Warner and Heimstra, 1973) the performance score on the second-to-last work period (period 10) was chosen as the dependent variable.

Putz *et al.* (1976) analyzed their data in terms of mean absolute deviation (MAD) scores. A more general analysis resulted from the use of logarithms of MAD scores (ln(MAD) scores). Appendix 2 contains the rationale for the logarithmic transformation which was used in Benignus *et al.*,(1987a) and in the analyses of the present study.

The confirmatory analysis was conducted with a linear model. The dependent variable was ln(MAD) tracking score for work period 10. The ln(MAD) tracking score from the first work period (pre-exposure) was included as a predictor to adjust for individual differences. The model could be thought of as an analysis of covariance, allowing unequal slopes and intercepts for control

and CO groups. The planned test of CO effect was the test of coincidence of the regression lines for prediction of work period 10 ln(MAD) scores from work period 1, for the control and CO groups. The alpha level for the test of coincidence was .05. This two-degrees-of-freedom test spans the test of equality of slopes (interaction of CO by the covariate) and the test of equality of intercepts (equality of covariate-adjusted means). If the test of coincidence were to be significant, the tests of equality of slopes and intercepts would be conducted as stepdown tests.

Exploratory Analyses. A number of additional analyses were planned. All such exploratory analyses were used for further hypothesis generation or, if appropriate, for supporting and further describing the outcome of the test of the *a priori* hypothesis. Any other exploratory analyses suggested by the results were also to be performed. No exploratory analyses were to be reported in the peer reviewed literature unless they fell within the guidelines of Muller, Barton and Benignus (1984).

Whether the test of the *a priori* hypothesis were to be significant or not, the data of all 10 work periods would be plotted and analyzed. Such analyses were to be made to explore the performance at times other than work period 10.

A frequency domain analysis was made of the human operator's tracking performance in terms of ccherence, gain and phase lag (Licklider, 1960) . The spectra were smoothed to gain stability of estimates by using equally weighted averages across four adjacent frequency bands (except for the second smoothed

frequency band which was averaged across only two bands). The averages were used to form a new spectrum with fewer frequency bands. The center frequencies in the smoothed spectrum range from .033 to .867 Hz, thereby encompassing the frequencies in the FF of .15 to .60 Hz. There were seven bands in the smoothed spectrum.

The EEG data were analyzed using power spectrum analysis to attempt to quantify changes in the subjects' state of alertness (Benignus 1984a). The EEG signal was split into one second segments, spectrum analyzed and then the spectra of the one second segments were averaged together to produce one power spectrum for each work period. A Hanning function had been applied to each one second segment before spectrum computation. Log power spectra were to be plotted and compared for the control and CO-exposed groups. Each spectrum was smoothed by averaging over adjacent frequency bands as described above so that the smoothed spectrum was composed of the four classical frequency bands; delta (1-4 Hz), theta (5-8 Hz), alpha (9-12 Hz) and beta (13-20 Hz). All power spectra were expressed as log 10 (power).

RESULTS

Table 3 gives the results of the COHb analyses on the subjects in this experiment. The means, standard deviations and ranges of COHb are given for the control and CO groups.

A Priori Hypothesis.

Table 4 gives the results of the test of the *a priori* hypothesis which tests for any difference in performance between control and CO groups. The test of coincidence, which includes a test of the equality of the covariate-adjusted means for the

control and CO groups, was not significant ($p>.397$). The test of slope differences was not significant ($p>.343$). Furthermore, the test of average slope was not significant ($p>.066$) indicating that the pre-exposure tracking score was not a useful covariate for the last work period. In this case the test of the intercept terms of the regression lines is equivalent to a test of the equality of the uncorrected means for the CO and control groups. This test was not significant ($p>.271$).

Exploratory Analyses

Further Tests of Work Period 10 Scores. Since the covariate was not needed in the above linear model, a slightly more powerful test of CO effect was an independent t test on work period 10 means. This test was also not significant ($t=-1.56$, $df=17$, $p>.137$). Table 5 gives the uncorrected means and standard deviations of the ln(MAD) scores of the CO and control groups for work periods 1-10.

Exploration of Tracking Scores. Figure 4 provides plots of ln(MAD) scores for work periods 1-10 with the ln(MAD) scores from work period 1 subtracted. Since means and variances are approximately constant for all work periods (see Table 5) and the correlations with the covariate score are always nearly 1.0, the difference scores are essentially equivalent to covariate adjusted scores. Since the difference scores are easier to present than covariate adjusted scores, they are used here.

The results of separate univariate tests for work periods 2-10 are shown in Table 6, using work period 1 scores as

covariates. Each row in table 6 provides an analysis for one work period, just as Table 4 provides the same analysis for work period 10. Hence, the last row of Table 6 duplicates p values from Table 4. The smallest p value for slope difference was p>.271 and for an intercept difference was p>.189. These results are consistent with the conclusion from the coincidence test for work period 10 (the work period in the *a priori* hypothesis test), *i.e.*, no effect of CO.

Average slopes were tested to evaluate the effectiveness of the covariate in each work period (see Table 6). The covariate was most influential on the work periods closest to it and least influential on the final work periods. However, inclusion of the covariate was at least marginally helpful in all work periods.

Other exploratory tests of interest were also performed. A test of coincidence of the average of work periods 2-10 was performed (F=.91, df=2 and 15, p>.423). A test of trends over the 10 work periods (first through eighth power) yielded no significant results (the smallest p value was for the linear trend, p>.166).

Spectrum Analysis of Tracking. Spectra of tracking behavior were computed on work period 10. Separate spectra were computed for CO and control groups. Figures 5-8 provide the power, coherence, gain and phase spectra for the CO and control groups. The equality of the CO and the control group spectra were tested using a two-way repeated measures analysis of variance with exposure, frequency and their interaction in the model. Table 7 gives the results of the four analyses. No test involving CO exposure reached the .05 level. The tests of the main effects of

frequency are tests of the flatness of the spectra. These are either "significant" or nearly so, implying that the tracking task is not equally well performed at all FF frequencies. Student's t tests for independent samples were performed at each frequency in each of the spectra. The results are shown in Table 8. Again no test involving CO reached the .05 level.

EEG Spectra. The power spectra of the EEG were computed for each of the work periods 1-10. The corresponding spectrum values from the first work period (pre-exposure) were subtracted from each of the spectrum values in work periods 2-10 to control for individual differences. Figure 9 is a plot of the delta band differences (from the first work period) across work periods 1-10 for the CO and the control groups. Figures 10-12 are the corresponding plots for the theta, alpha and beta bands, respectively. Tables 9-12 give the uncorrected means and standard deviations over the 10 work periods for both CO and control groups for the four EEG spectrum bands.

Within-band difference scores were chosen as dependent variables for the EEG data since work period 1 spectrum values correlated highly (approximately .90) with spectrum measures on all other blocks and since variances were nominally constant across all 10 blocks, within each band. A multivariate analysis of variance testing exposure effect was performed on the difference scores of work periods 2-10, minus work period 1 separately for each band. The results are shown in Table 13. None of the four frequency bands showed significant differences between control and CO groups. The smallest value was $p > .221$.

Separate t tests of exposure for each work period difference for each frequency band were also computed (see Table 14). As can be expected when performing 40 t tests on related samples, a few achieved the .05 level, but no plausible indication of a CO effect can be seen.

Predictions of COHb Levels Throughout the Study. The results from Appendix 1 may be summarized as follows. COHb levels were observed only for pre- and post-exposure. Pilot work appeared to indicate that the exposure methods were producing the desired results. After completion of the entire experiment, mathematical predictions were used to estimate the COHb levels at times in the study when no measurement was available. This extrapolation led to the conclusions that the subjects' COHb levels continued to increase in a slow manner throughout the experiment, and that COHb must have been lower than predicted at the end of bolus exposure. No actual evidence is available to resolve the problem. Regardless of whether the COHb was constant throughout the experiment, the COHb at the end of the experiment was near the intended value.

Statistical Power Analysis. If statistical tests are non-significant, the question arises as to whether the statistical power (sensitivity) of the tests was sufficiently great to have allowed detecting differences of interesting size, with the given sample size, observed variance and selected alpha level. The most sensitive test which still accounts for individual differences was the t test of the difference-score-corrected work period 10 means.

The observed variances for the corrected performance scores

and sample sizes from the present study were used with an alpha of .05 to conduct a statistical power analysis (Muller and Peterson, 1984). Figure 13 is a plot of the statistical power estimated as a function of the possible sizes of the difference between the mean corrected performance scores of the CO and control groups. The size of the postulated difference is expressed in units of ln(MAD) scores. Also shown in Figure 13 are the points on the curve corresponding to the observed differences in mean corrected performance scores for the present study, the study by Putz _et al._ (1976) and the study by Benignus _et al._ (1987a). From Figure 13 it may be seen that the present experiment had a power approaching 1.0 for differences as large as those observed with slow COHb formation by Putz _et al._ at 5% COHb and a power of .44 for differences as large as observed by Benignus _et al._ at 8% COHb. Power for the size of the difference observed in the present study using rapid COHb formation was, of course, extremely low (.09).

DISCUSSION

Lack of Effects of COHb.

The COHb levels which had formed by the end of the study (16.4 - 22.8%) were much larger than those of previous studies of 5% (Putz _et al._, 1976, 1979) or 8% (Benignus _et al._, 1987a). The earlier studies demonstrated statistically significant effects on a similar test. The COHb levels were near the point at which symptoms of CO poisoning are said to occur and oxygen therapy is usually initiated (Friedman, 1980; Klingberg, 1983; Klaassen, 1983; Goldfrank _et al._, 1986).

Despite the high levels of COHb, there were no significant effects on the tracking task. Even for very liberal *post hoc* tests, no hint of a significant effect emerged. Yet the power of the most sensitive significance test was moderate to high for effects which were seen by Putz *et al.* at 5% COHb. Thus the failure to find significant results cannot be explained as due to a test which was insensitive to effects of interesting size, especially for so large a level of COHb. The spectrum method of analysis of the tracking behavior did not show any effects due to COHb. The latter method would be expected to be more sensitive than analysis of MAD scores because effects occurring only in *e.g.* the high frequency band would be isolated from the random variations in the other frequency bands.

The fact that tests of significance which were powerful against sizes of effects seen by Putz *et al* at 5% COHb, did not demonstrate any effect of COHb on behavior, even for large values of COHb, is somewhat surprising. The EEG was not significantly affected by the COHb values either. For whatever reason, there appear to be conditions under which COHb values, usually considered to be patently dangerous, do not affect some behaviors or electrophysiological measures. This conclusion is supported by the observation that these and the pilot subjects did not report significantly more symptoms of CO poisoning at high COHb levels (Benignus *et al.*, 1987b). Clearly, a number of aspects of CO effects are not understood.

Possible Explanations for the Findings.

Task and Procedural Variables. Several aspects of the present experiment were different from earlier studies which

produced significant results with lower levels of COHb. It is, therefore, possible that a critical variable was changed which made the experiment insensitive to COHb effects. The following are possibilities.

The tracking task used a FF which was much more difficult to track than in previous studies. This was due to the higher frequency components (greater spot speeds) as well as to a more unpredictable spot motion. According to the findings of Putz *et al.* (1976, 1979) increased difficulty should make the task more sensitive to disruption by COHb. Perhaps too much difficulty was achieved, however, thus placing the difficulty of the task near the upper end of the sensitivity curve (Benignus, 1984b).

The timing of the work/rest cycle in the present study was different from that of previous work. In the previous studies, the subject performed for a longer period of time during each hour, but there were more short breaks within the period of performance. The total time in the chamber on previous studies was also longer than for the present study. If the effect of COHb is upon perceptual-motor skills, as seems likely, then the particular work/rest cycle should not be so great a variable as to so drastically change task sensitivity.

The earlier experiments used a divided attention task in the form of a foreground (tracking) task with a background (light monitoring) task. If the effect of COHb were to be a reduction of the ability to perform such tasks, *e.g.* reduction in time sharing speed or reduction in channel capacity, the elimination of the background light monitoring task could have reduced the

sensitivity of the experiment.

It may be argued for any of the above reasons that the behavioral task was not sensitive to COHb effects. The spectrum of the EEG is, however, not task specific and it too was unaffected by the COHb levels. Even if the behavioral measure was insensitive, it would seem that a COHb level as high as was used in this study would have produced general enough effects to have altered the EEG. The lack of EEG effects is consistent with the conclusion that there were no significant effects of COHb on these subjects and that the lack of significance was not an artifact of the particulars of the design of the experiment.

Exposure Variables. Another major difference in the present study with respect to earlier work is the rate at which COHb was formed. Earlier work used low level CO exposure so that the COHb level continued to increase slowly throughout the experiment. In the present study, a bolus of CO near the beginning of the experiment raised the COHb quickly and a low maintenance CO level kept the COHb relatively constant for the remainder of the time. Although there are some exceptions, the assumption is usually made (Coburn, 1979) that the COHb level and not the rate of COHb formation is the critical variable in CO effects since COHb is related to the ability of the blood to carry oxygen per fixed volume of blood flow.

Substantial vasodilation occurs in the brains of dogs as a function of COHb level (Traystman et al., 1977; Traystman, 1978). Such vasodilation tends to increase blood flow and thereby deliver more oxygen to the brain. The response time of this phenomenon is unknown since it has only been studied with bolus

exposure. If the vasodilation response only occurred in the presence of rapid changes of COHb, but not in the presence of slow changes, then the CNS effect of bolus exposure would be reduced with respect to slow COHb formation. It is not an unreasonable assumption that a "compensatory" mechanism such as brain vasodilation would be more responsive to sudden changes in COHb than to slow changes. There exist many examples of sensors in physiology which are essentially differentiators.

Dose Effects Function. Another possible explanation for the findings of the present study is that the dose effects function of COHb is not monotonic. If the data from the earlier work are combined with the data from the present study and if the COHb formation rate is ignored, then it appears that the effect size diminishes as COHb increases between the limits of 5% and about 18% (see Figure 4). No dose effects data for the behavior in question is available, so the hypothesis of a non-monotonic function is pure speculation.

Spectra of Tracking Behavior.

The spectra of the tracking behavior provide clues to the nature of the task performance errors. From Figure 5 it may be seen that the power of the joystick pressure history was smaller than the power in the FF at all frequencies. This implies that the subjects were always under-compensating the spot movement. There was more under-compensation at higher than at lower frequencies.

The coherence spectrum indicates that the joystick motion was a faithful replica of the FF because the coherences

(analogous to squared correlations) are very high at each frequency band. Apparently there was little tremor or other irrelevant motion. The gain spectrum values are less than unity at all frequencies. Since the FF and the joystick pressure history were equally scaled and coherences were near unity, the less than unity gain implies that the subjects were not sufficiently sensitive to the FF. As with the power spectrum, it appears that the sensitivity of the subject increased as a function of the frequency of the FF. Inspection of the phase spectrum leads to the conclusion that the joystick response always lags the FF by 75 to 750 ms. depending upon the exposure condition and the frequency of the FF. In general, the higher the frequency, the greater the lag of the joystick response.

Conclusion.

The findings of no significant effects of high COHb levels is somewhat surprising and potentially important. Much information would be gained about the mechanism of COHb effects if the effects were shown to be mitigated by task parameters or rate of COHb formation. It would be a matter of both regulatory and theoretical concern if the dose effects function of COHb were not monotonic. Any or a combination of the above speculations may be true. Not enough data of the right kind are presently available to choose among these speculations.

LITERATURE CITED

Bendat, J.S. and Piersol, A.G. 1966. Measurement and Analysis of Random data. New York, John Wiley.

Benignus, V.A. 1984a EEG as a cross species indicator of neurotoxicity. Neurobehav. Toxicol. Teratol. 6:473-483.

Benignus, V.A. 1984b. Das bewirkungen Kohlen Monoxid am Gehirn. Seminar presented at the Whermedizin Institut, Koblenz, W. Germany, October, 1984.

Benignus, V.A., Muller, K.E., Barton, C.N., Prah, J.D. and Benignus, L.L. 1983. Neurobehavioral Effects of Carbon Monoxide (CO) Exposure in Humans. Final Report. U.S. Army Medical Bioengineering Research and Development Command, Ft. Detrick, Frederick, MD. Publication No. AD-A133891.

Benignus, V.A., Muller, K.E., Barton, C.N. and Prah, J.D. 1987a. Effects of low level carbon monoxide on compensatory tracking and event monitoring. Neurobehav. Toxicol. Teratol. In Press.

Benignus, V.A., Kafer, E.R., Muller, K.E. and Case, M.W. 1987b. Absence of symptoms with carboxyhemoglobin levels of 16-23%. In Review.

Bosser, T. 1984. Adaptation to time-varying signals and control-theory models of tracking behavior. Psychological Res. 46:155-167.

Friedman, P.A. 1980. Common poisons. In: Isselbacher, K.J., Adams, R.D., Braunwald, E., Petersdorf, R.G. and Wilson, J.D., Eds. Harrison's Principles of Internal Medicine, 9th Ed., New York, Mcgraw-Hill, 953-964.

Coburn, R.F., Forster, R.E. and Kane, P.B. 1965. Considerations

of the Physiological variables that determine the blood carboxyhemoglobin concentration in man. J. Clin. Invest. 44:1899-1910.

Coburn, R.F. 1979. Mechanisms of carbon monoxied toxicity. Prev. Med. 8310-322.

Goldfrank, L.R., Lewin, N.A., Kerstein, R.H. and Weisman, R.S. 1986. Carbon monoxide. In: Goldfrank, L.R., Flomenbaum, N.E., Lewin, N.A., Weisman, R.S., Howland, M.A. and Kulberg, A.G., Eds. Goldfrank's Toxicologic Emergencies. 3rd Ed., Norwalk, CT, Appelton-Century-Crofts, 662-671.

Johnson, N.L. and Kotz, S. 1971. Continuous Univariate Distributions, Vol. 1, New York, John Wily.

Klaassen, C.D. 1985. Nonmetallic toxicants: Air pollutants, solvents, vapors and pesticides. In: Goodman, A.G. and Gillman, A., Eds. The Pharmocological Basis of Therapeutics. New York, MacMillan, 1628-1650.

Klingberg, C.A. 1983. Carbon monoxide poisoning. In: Hurst, J.W., Ed. Medicine for the Practicing Physician. Boston, Butterworths, 1849-1853.

Licklider, J.C.R. 1960. Quasi-linear models in the study of manual tracking. In: Luce, R.D., Developments in Mathematical Psychology. Glencoe, IL. Free Press of Glencoe, 171-280.

Muller, K.E., Barton, C.N. and Benignus, V.A. 1984. Recommendations for appropriate statistical practice in toxicologic experiments. Neurotoxicol. 5:113-126.

Muller, K.E. and Peterson, B.L. 1984. Practical methods for

computing power in testing the multivariate general linear hypothesis. Computat. Stat. Data Anal. 2:143-158.

Muller, K.E. and Barton, C.N. 1987. Fitting a nonlinear version of Coburn, Forster Kane model of blood carboxyhemoglobin. Atmospheric Environ. In Press.

Notterman, J.M. and Weitzman, D.O. 1981: Organizatiion and learning of visual-motor information during different orders of limb movement: Step, velocity, acceleration. J. Exp. Psychol. Human Percept. Perform. 7:916-927.

Putz, V.R. Johnson, B.L. and Setzer, J.V. 1976. Effects of CO on Vigilance Performance. U.S. Dept of Health Education and Welfare, National Institute for Occupational Safety and Health,Cincinnati, OH. DHEW (NIOSH) Publication No. 77-124.

Putz, V.R. Johnson, B.L. and Setzer, J.V. 1979. A comparative study of the effects of carbon monoxide and methlyene chloride on human performance. J. Environ Pathol. Toxicol. 2:97-112.

Stewart, R.D., Peterson, J.E., Fisher, T.N., Hosko, M.J., Baretta, E.D., Dodd, H.C. and Herrman, A.A. 1973. Experimental human exposure to high concentrations of carbon monoxide. Arch. Env. Health. 26:1-7.

Traystman, R.J. and Fitzgerald, R.S. 1977. Cerebral circulatory responses to hypoxic hypoxia and carbon monoxide hypoxia in carotid barorecptor denervated dogs. Acta Neurol. Scand. suppl. 64:294-295.

Traystman, R.J. 1977. Effect of carbon monoxide hypoxia and hypoxic hypoxia on cerebral circulation. In: Otto, D.A. Ed., Multidisciplary Perspectives in Event-Related Brain

Potential Research. Washington, DC. U.S. Environmental Protection Agency publication No. EPA-600/9-77-043, 453-457.

Warner, D.W. and Heimstra, N.W. 1973. Target-detection performance as a function of noise intensity and task difficulty. *Percept Mot. Skills.* 36:439-442.

GLOSSARY

a priori	Before the fact, usually referring to an experimental hypothesis made before conducting a study.
Bonferroni Correction	A statistical technique employed to adjust the alpha level (p value which will be acceptable as a significant outcome of a statistical test). Such adjustment is needed when a number of significance tests are performed on a body of related variables.
Coherence Spectra	See "Spectrum"
Double blind	An experimental strategy in which neither the subject nor the experimenter in contact with the subject is informed of the exposure condition. The strategy is used to minimize experimenter and subject bias due to expectation.
Forcing function	A time varying voltage or function used to drive the moving spot away from the target on an oscilloscope screen.
Gain Spectra	See "Spectrum".
Joystick	a lever similar to that used in control apparatus or in video games to manually manipulate some variable.
Phase Spectra	See "Spectrum".
post hoc	After the fact, in this text, refers to some analysis or deduction made after the data has been inspected.
Power Spectra	See "Spectrum".
Power (Statistical)	Probability of correctly detecting an effect which is present in the population.
Spectrum	The frequencies of a series of waves which compose a signal. Power spectra specify the energy level in each frequency. Coherence spectra specify the strength of relationship between waves at each frequency.Gain spectra specify the sensitivity of a system to each frequency. Phase spectra specify the temporal relations between two waves ateach frequency.
Tracking	A task in which the subject must respond to a display to either pursue a target (pursuit tracking) or to compensate for the motion of a target (compensatory tracking).

TABLE 1. SCHEDULE OF EVENTS ON DAY OF EXPERIMENT

Activity	Cumulative Time
Informed consent, blood draw	00
Begin training in chamber	15
Apply electrodes after training	85
Start experiment	105
Finish experiment	235

TABLE 2. ORDER OF FF SEGMENTS USED IN EACH WORK PERIOD

Work Period	FF Number				
1	1	2	3	4	5
2	2	3	1	8	9
3	3	1	2	9	10
4	6	9	7	10	3
5	5	8	6	3	7
6	4	7	5	6	2
7	10	6	4	2	1
8	9	5	10	1	8
9	8	4	9	7	6
10	7	10	8	5	4
11	1	2	3	4	5

TABLE3. COHbLEVELS(%)BEFOREANDAFTER EXPOSURE

	Group	N	Mean	± S.D.	Range
Pre Exposure	Control	10	1.1	.4	.7 - 1.9
	CO	9	1.4	.3	.9 - 1.8
Post Exposure	Control	10	1.0	.3	.6 - 1.6
	CO	9	18.3	2.1	16.4 - 22.8

TABLE 4. RESULTS OF THE TEST OF THE A PRIORI HYPOTHESIS USING A TEST OF COINCIDENCE

Source of Variance	F	df 1	df 2	p value
Coincidence[1]	.98	2	15	.397
Intercept[2]	1.30	1	15	.271
Slope[3]	.96	1	15	.343
Average Slope[4]	3.93	1	15	.066

1 The test of coincidence is a test of the equality of the two regression lines predicting work period 10 performance scores from performance scores of work period 1 between control and CO groups. The coincidence test is a simultaneous test of slope and intercept.

2 The test of the intercept is a test of the difference in intercepts between control and CO groups.

3 The test of slope is a test of the difference in slopes between control and CO groups.

4 The test of average slope evaluates whether the covariate improves the model, given that the slopes are equal.

TABLE 5. UNCORRECTED MEANS AND STANDARD DEVIATIONS OF THE ln(MAD) SCORES FOR WORK PERIODS 1-10 FOR CO AND CONTROL GROUPS

CO Group			Control Group		
Work Period	Mean	± S.D.	Work Period	Mean	± S.D.
1	.699	.131	1	.625	.110
2	.671	.112	2	.621	.119
3	.676	.121	3	.596	.124
4	.659	.139	4	.578	.093
5	.647	.140	5	.533	.084
6	.700	.135	6	.600	.124
7	.696	.130	7	.586	.129
8	.652	.129	8	.540	.114
9	.680	.163	9	.574	.091
10	.651	.179	10	.543	.120

TABLE 6. EXPLORATORY P VALUES FOR F TESTS OF THE MODEL DESCRIBED IN TABLE 4 WITH BLOCK 1 AS A COVARIATE AND BLOCKS 2-10 AS DEPENDENT VARIABLES

WorkPeriod	Coincidence[1]	Slope[2]	Intercept[2]	Average Slope[2]
2	.801	.534	.566	$7x10^{-5}$
3	.766	.650	.585	.0005
4	.732	.699	.795	.0007
5	.319	.832	.949	.0017
6	.450	.405	.325	.0033
7	.402	.455	.352	.0011
8	.225	.271	.189	.0132
9	.380	.453	.345	.0759
10	.397	.343	.271	.0660

[1] Each test had 2 and 15 degrees of freedom.

[2] Each test had 1 and 15 degrees of freedom.

TABLE 7. TWO-WAY REPEATED MEASURES ANALYSES OF VARIANCE[1] AS EXPLORATORY TESTS OF THE EQUIVALENCE OF CO AND CONTROL SPECTRA OF TRACKING BEHAVIOR.

Dependent Variable	Source of Variance	F	E[2]	df	p value[3]
Power	Frequency x CO	.27	.51	3,51	.703
	Frequency	6.26	.51	3,51	.010
	CO	.11	-	1,17	.740
Coherence	Frequency x CO	.43	.47	3,51	.587
	Frequency	.67	.47	3,51	.473
	CO	.12	-	1,17	.728
Gain	Frequency x CO	.49	.47	3,51	.557
	Frequency	6.66	.47	3,51	.010
	CO	.05	-	1,17	.820
Phase	Frequency x CO	.90	.45	3,51	.384
	Frequency	3.10	.45	3,51	.081
	CO	.00	-	1,17	.992

1 Dependent variable was ln(MAD) on work period 10 with ln(MAD) on work period 1 subtracted.

2 E is the estimated Geisser-Greenhouse correction.

3 p values are Geisser-Greenhouse corrected, except for CO main effects, which are from independent groups.

TABLE 8. EXPLORATORY T TESTS[1,2] OF DIFFERENCES BETWEEN CO AND CONTROL FOR EACH FREQUENCY BAND IN EACH SPECTRUM OF TRACKING BEHAVIOR

Dependent Variable	Frequency	p value	Dependent Variable	Frequency	p value
Power	.083	.108	Coherence	.083	-
	.183	.273		.183	.203
	.283	.230		.283	.064
	.417	.188		.417	.053
	.550	.128		.550	.143
	.683	.381		.683	-
	.817	.526		.817	-
Gain	.083	-	Phase	.083	-
	.183	.253		.183	.514
	.283	.205		.283	.647
	.417	.161		.417	.756
	.550	.119		.550	.647
	.683	-		.683	-
	.817	-		.817	-

[1] Each test has 17 degrees of freedom.

2 Tests were not conducted when power in a particular frequency band approached zero.

TABLE 9. UNCORRECTED MEANS AND STANDARD DEVIATIONS FOR THE DELTA BAND OF THE EEG OVER THE 10 WORK PERIODS FOR CO AND CONTROL GROUPS

CO Group			Control Group		
Work Period	Mean	± S.D.	Work Period	Mean	± S.D.
1	-.247	.280	1	-.097	.153
2	-.257	.266	2	-.091	.130
3	-.249	.237	3	-.071	.131
4	-.263	.228	4	-.076	.121
5	-.244	.292	5	-.101	.131
6	-.236	.272	6	-.074	.130
7	-.268	.263	7	-.065	.136
8	-.224	.365	8	-.041	.207
9	-.173	.355	9	-.054	.180
10	-.248	.263	10	-.055	.153

TABLE 10. UNCORRECTED MEANS AND STANDARD DEVIATIONS FOR THE THETA BAND OF THE EEG OVER THE 10 WORK PERIODS FOR CO AND CONTROL GROUPS

CO Group			Control Group		
Work Period	Mean	± S.D.	Work Period	Mean	± S.D.
1	-.402	.189	1	-.237	.125
2	-.376	.223	2	-.257	.135
3	-.388	.221	3	-.257	.120
4	-.393	.172	4	-.260	.143
5	-.387	.188	5	-.264	.146
6	-.355	.217	6	-.233	.139
7	-.362	.191	7	-.205	.119
8	-.351	.220	8	-.172	.226
9	-.370	.202	9	-.174	.239
10	-.377	.185	10	-.136	.209

TABLE 11. UNCORRECTED MEANS AND STANDARD DEVIATIONS FOR THE ALPHA BAND OF THE EEG OVER THE 10 WORK PERIODS FOR CO AND CONTROL GROUPS

CO Group			Control Group		
Work Period	Mean	± S.D.	Work Period	Mean	± S.D.
1	-.314	.306	1	.064	.317
2	-.232	.294	2	.058	.349
3	-.281	.323	3	.053	.341
4	-.307	.294	4	.079	.343
5	-.326	.323	5	.079	.344
6	-.320	.312	6	.119	.357
7	-.316	.323	7	.133	.383
8	-.317	.322	8	.160	.351
9	-.341	.307	9	.149	.362
10	-.311	.296	10	.143	.342

TABLE 12. UNCORRECTED MEANS AND STANDARD DEVIATIONS FOR THE BETA BAND OF THE EEG OVER THE 10 WORK PERIODS FOR CO AND CONTROL GROUPS

CO Group			Control Group		
Work Period	Mean	± S.D.	Work Period	Mean	± S.D.
1	-.936	.284	1	-.682	.254
2	-.892	.315	2	-.743	.190
3	-.915	.325	3	-.768	.200
4	-.934	.291	4	-.768	.188
5	-.915	.314	5	-.759	.174
6	-.954	.297	6	-.741	.188
7	-.954	.280	7	-.705	.190
8	-.943	.287	8	-.614	.367
9	-.947	.297	9	-.649	.381
10	-.916	.302	10	-.633	.347

TABLE 13. EXPLORATORY MULTIVARIATE ANALYSES OF VARIANCE[1] TESTING DIFFERENCES BETWEEN CO AND CONTROL SPECTRA OF EEG.[2]

Frequency Band	Wilks' Lambda	F	p value
Delta	.24	1.91	.221
Theta	.64	.34	.938
Alpha	.27	1.60	.292
Beta	.41	.85	.611

1 Degrees of freedom for each test are 10 and 6.

2 Dependent variables were values for work period 10 with values of work period 1 subtracted.

TABLE 14. EXPLORATORY T TESTS[1] FOR EACH EEG FREQUENCY BAND, TESTING THE DIFFERENCE BETWEEN CO AND CONTROL[2] AT EACH WORK PERIOD

Frequency Band	Work Period	t	p value	Frequency Band	Work Period	t	p value
Delta	2	.48	.641	Alpha	2	-1.62	.126
	3	1.25	.230		3	-0.69	.500
	4	1.23	.238		4	.15	.884
	5	-0.26	.797		5	.42	.678
	6	.56	.584		6	1.06	.304
	7	1.96	.068		7	.88	.395
	8	.14	.891		8	1.00	.333
	9	-1.08	.296		9	1.30	.214
	10	1.32	.208		10	.55	.587
Theta	2	-1.45	.169	Beta	2	-1.97	.068
	3	-0.95	.356		3	-2.17	.047
	4	-0.91	.379		4	-1.42	.176
	5	-1.28	.219		5	-1.21	.244
	6	-1.28	.221		6	-0.64	.529
	7	-0.97	.347		7	-1.12	.281
	8	-1.09	.292		8	-0.57	.575
	9	-0.66	.520		9	-1.46	.165
	10	.21	.834		10	-1.83	.088

[1] Each test has 15 degrees of freedom.

[2] Dependent variables were values from work period 2-10 with values from work period 1 subtracted.

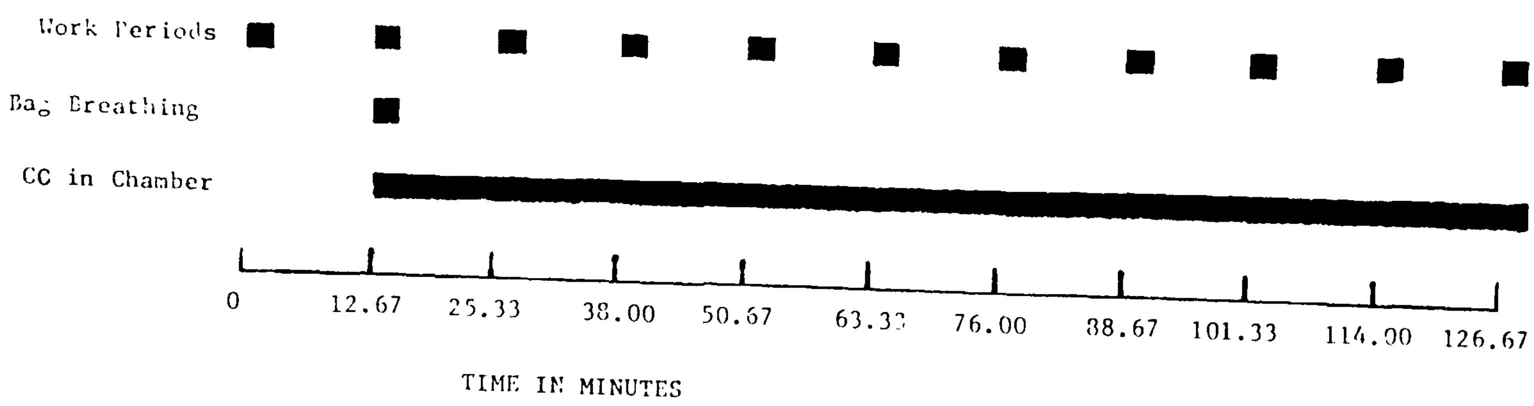

Figure 1. Graph of the events during the course of the approximately 130 minute experiment.

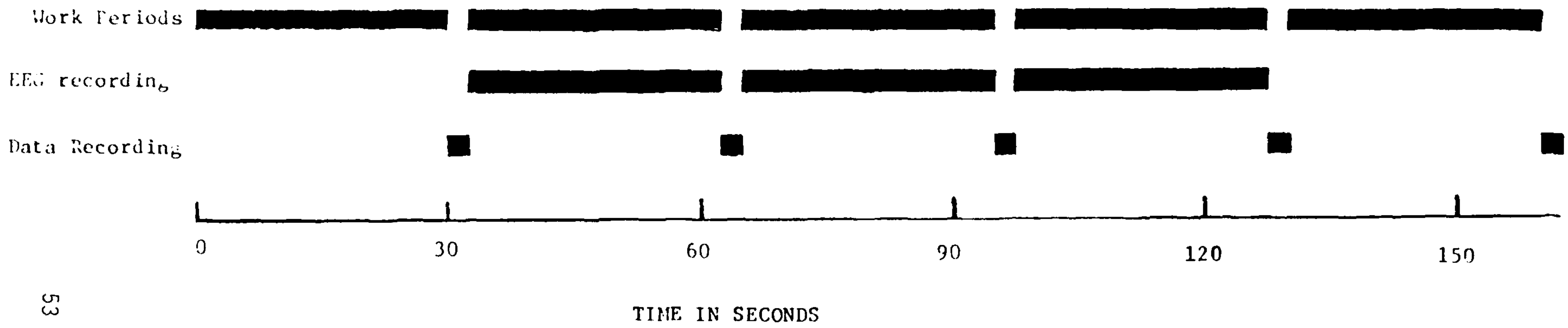

Figure 2. Graph of the events during a single work period.

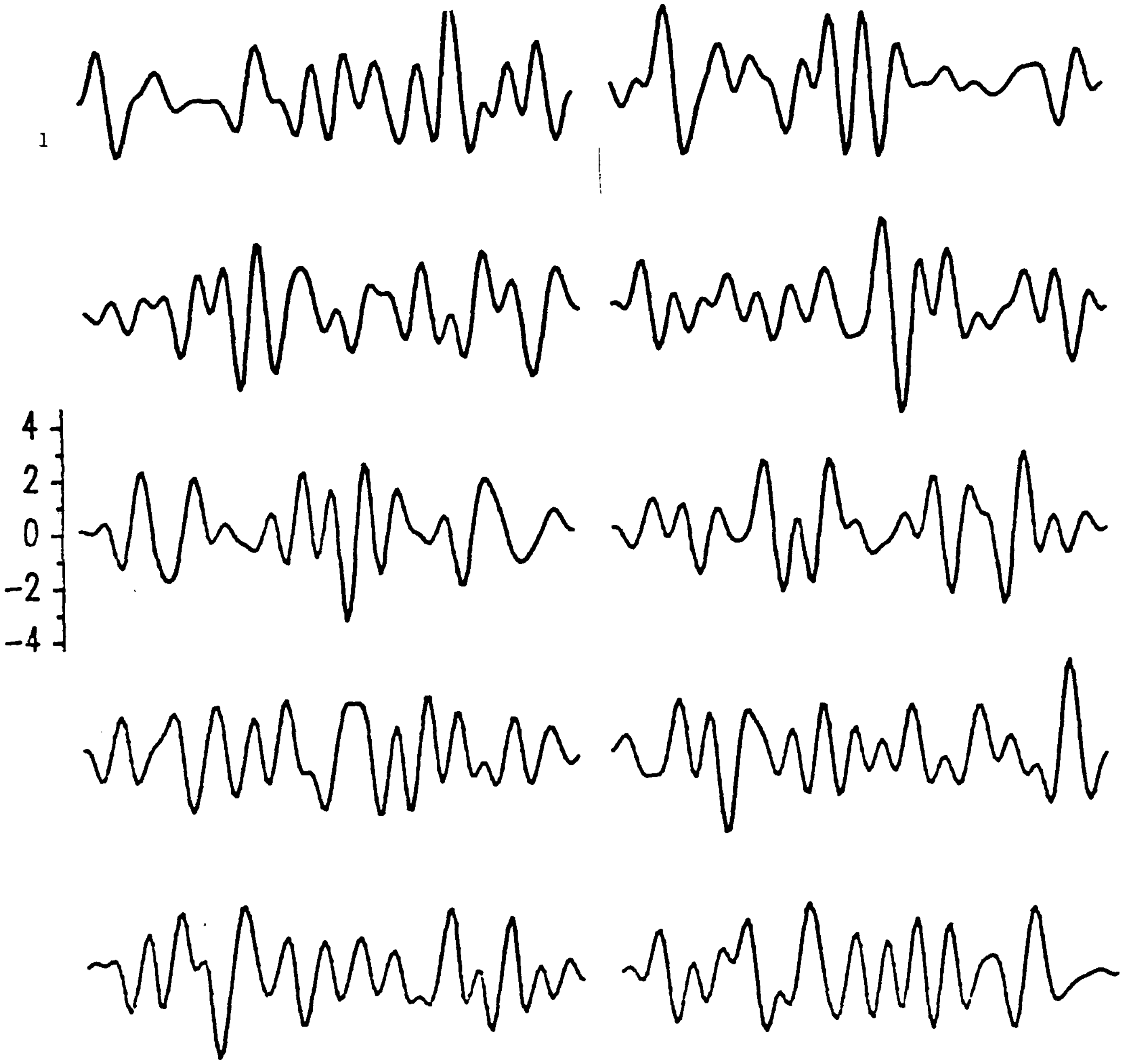

Figure 3 Plots of the 10 forcing function segments which were used to construct the 2.67 minute forcing functions for each work period.

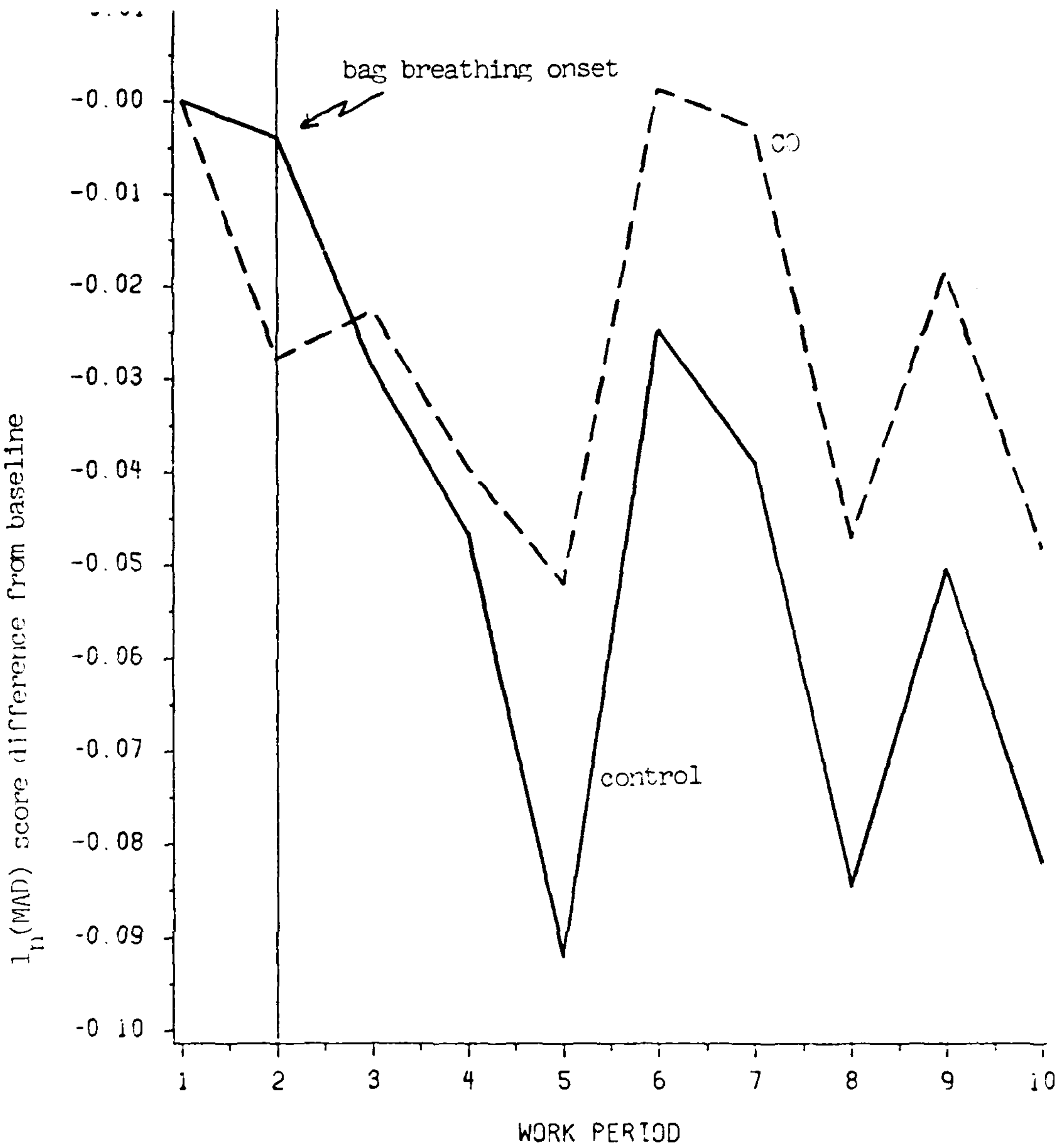

Figure 4. Plots of ln(MAD) scores for work periods 1-10, for both CO and control groups. Each point represents the ln(MAD) score for that work period with the ln(MAD) score from the first work period (pre exposure) subtracted.

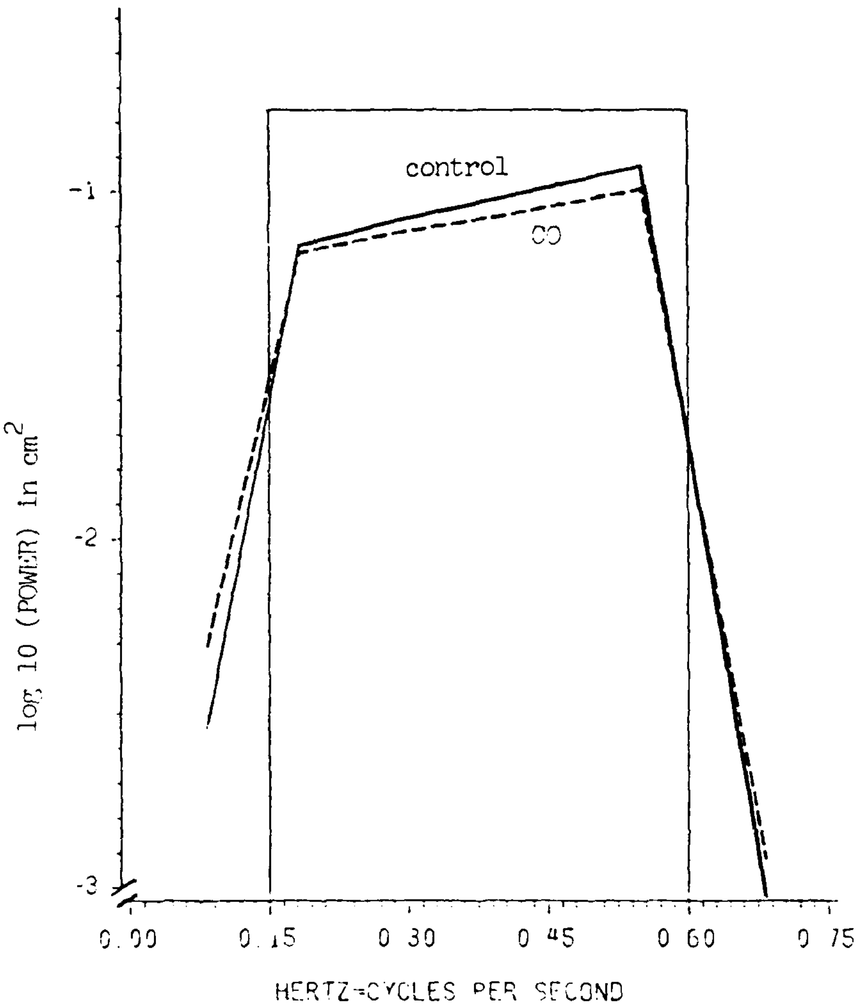

Figure 5. Log10 (Power) spectra of tracking behavior in work period 10 for CO and control groups. The box shaped spectrum in the figure is the spectrum of the FF.

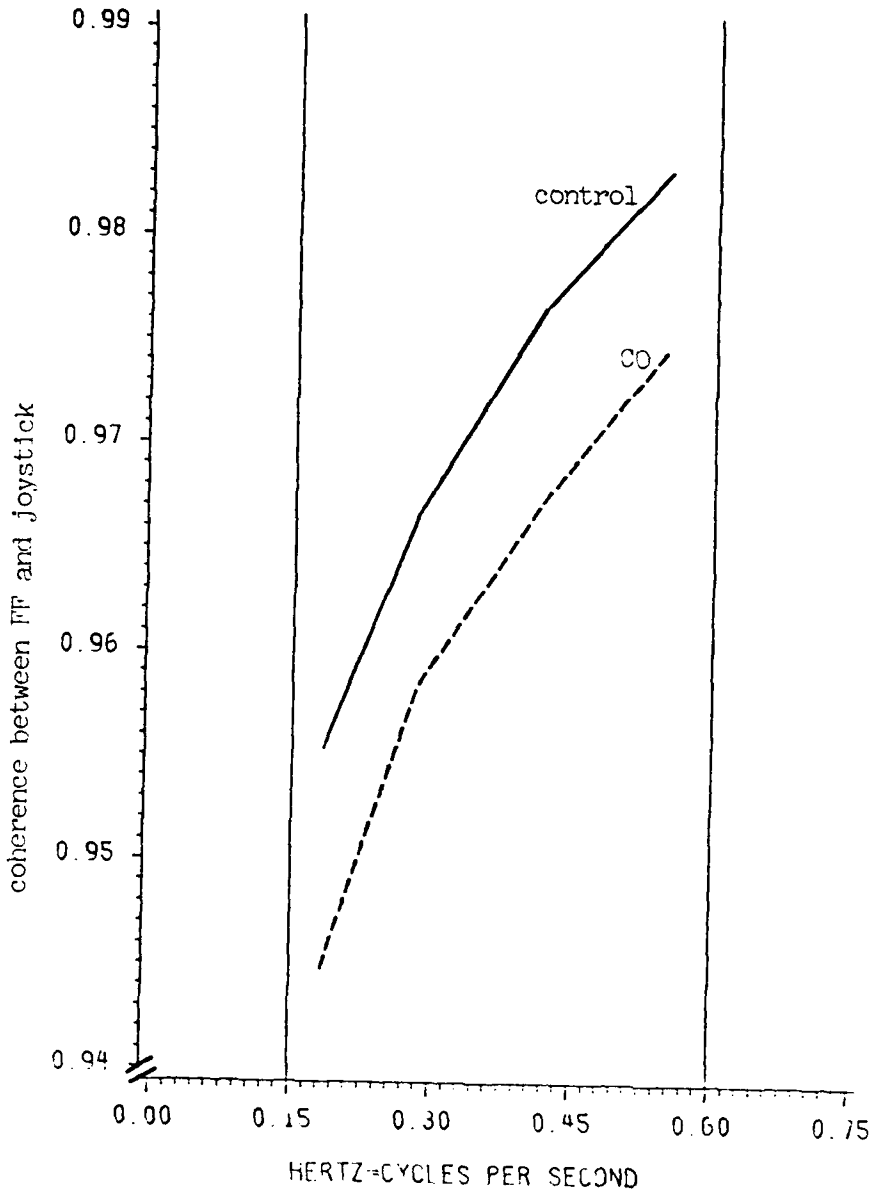

Figure 6. Coherence spectra of tracking behavior in work period 10 for CO and control groups. The vertical lines in the figure indicate the limits of the FF spectrum.

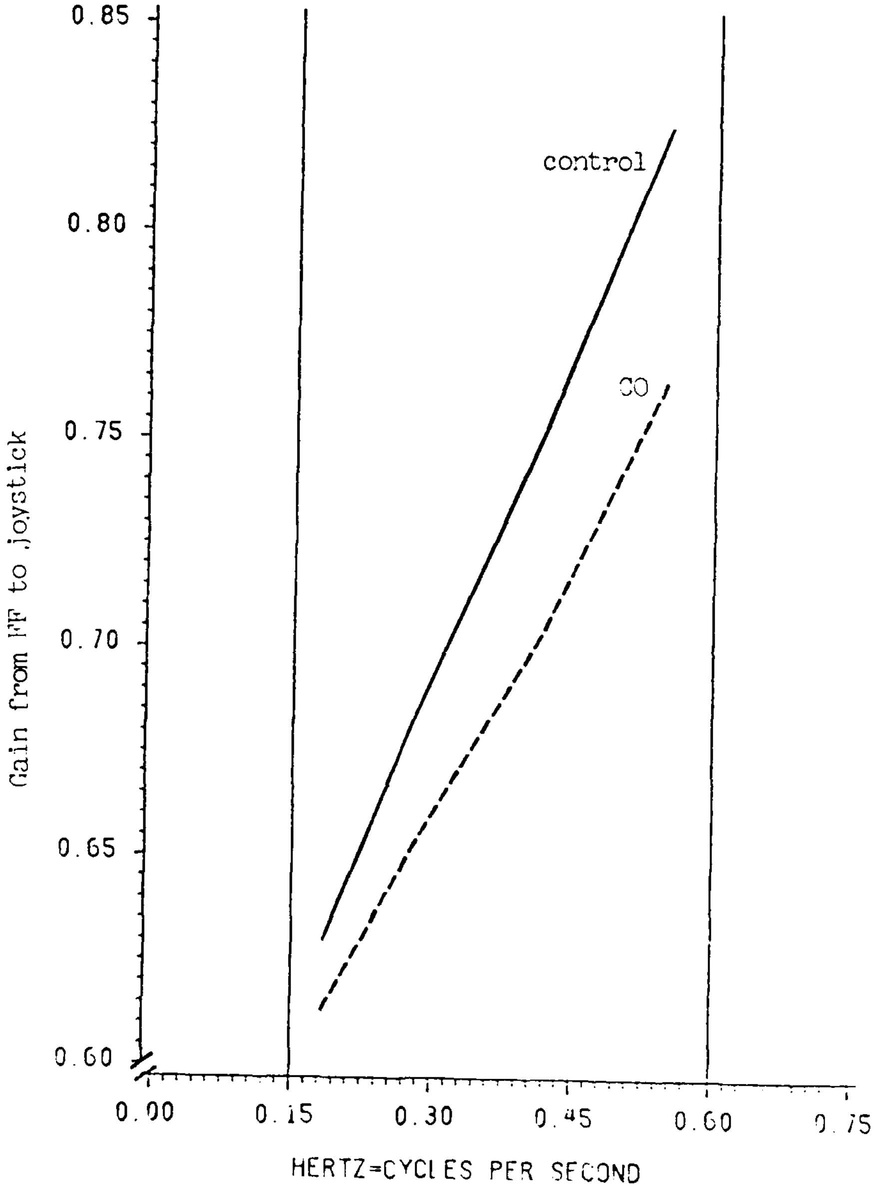

Figure 7. Gain spectra of tracking behavior in work period 10 for CO and control groups. The vertical lines in the figure indicate the limits of the FF spectrum.

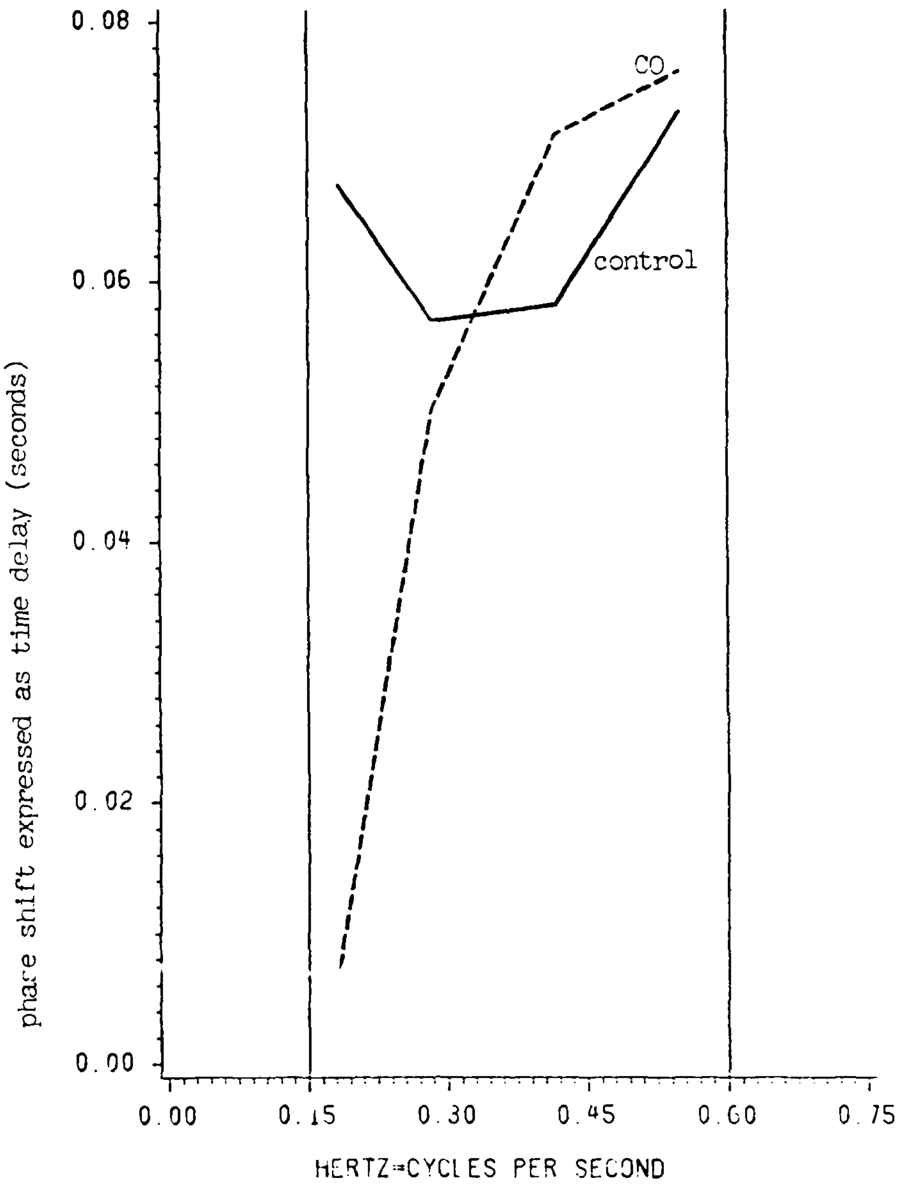

Figure 8. Phase spectra of tracking behavior in work period 10 for CO and control groups. The vertical lines in the figure indicate the limits of the FF spectrum. Phase is expressed as time delay in seconds.

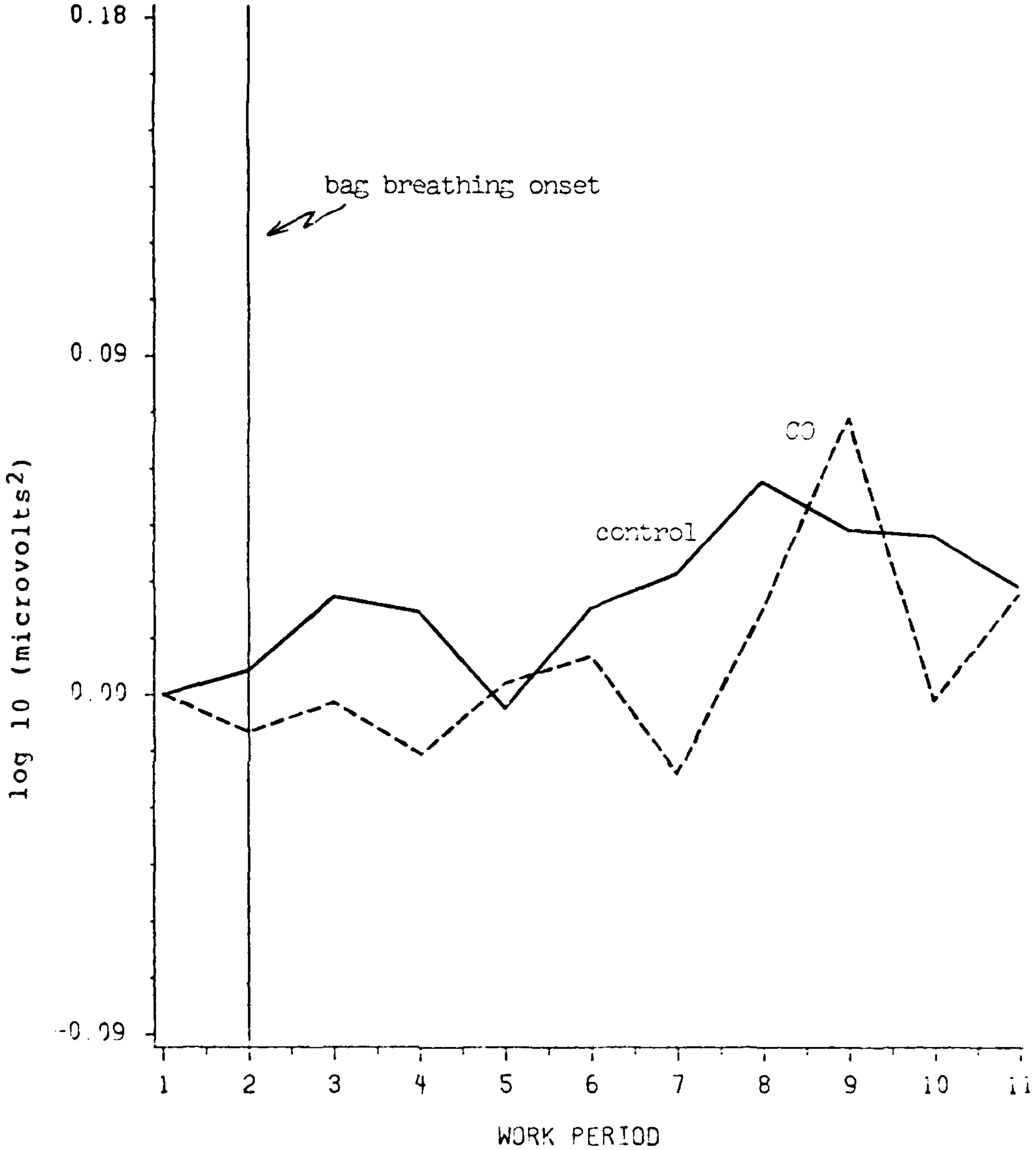

Figure 9. Difference in EEG delta power from first work period (pre exposure), for work periods 1-10, for CO and control groups.

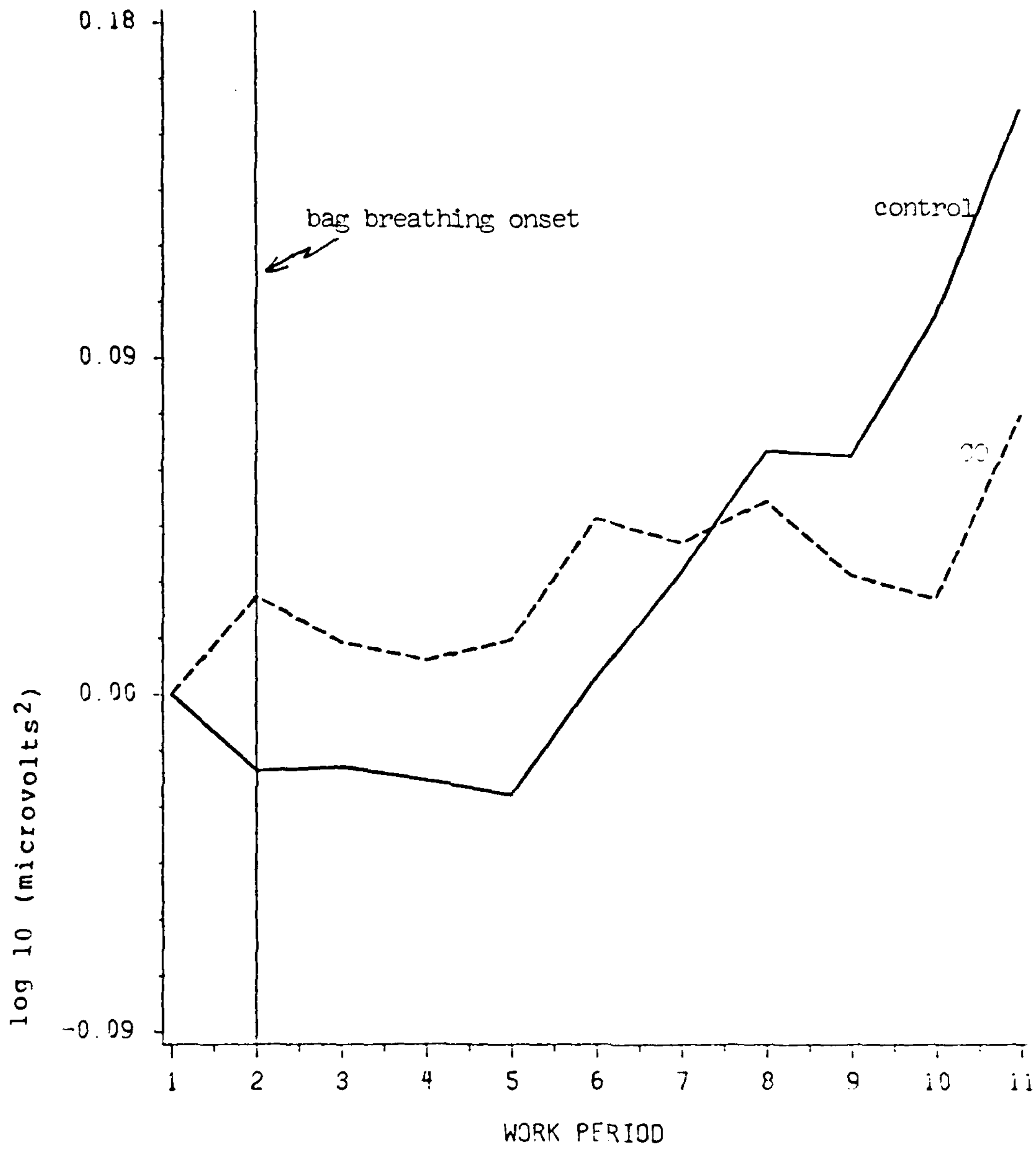

Figure 10. Difference in EEG theta power from first work period (pre exposure), for work periods 1-10, for CO and control groups.

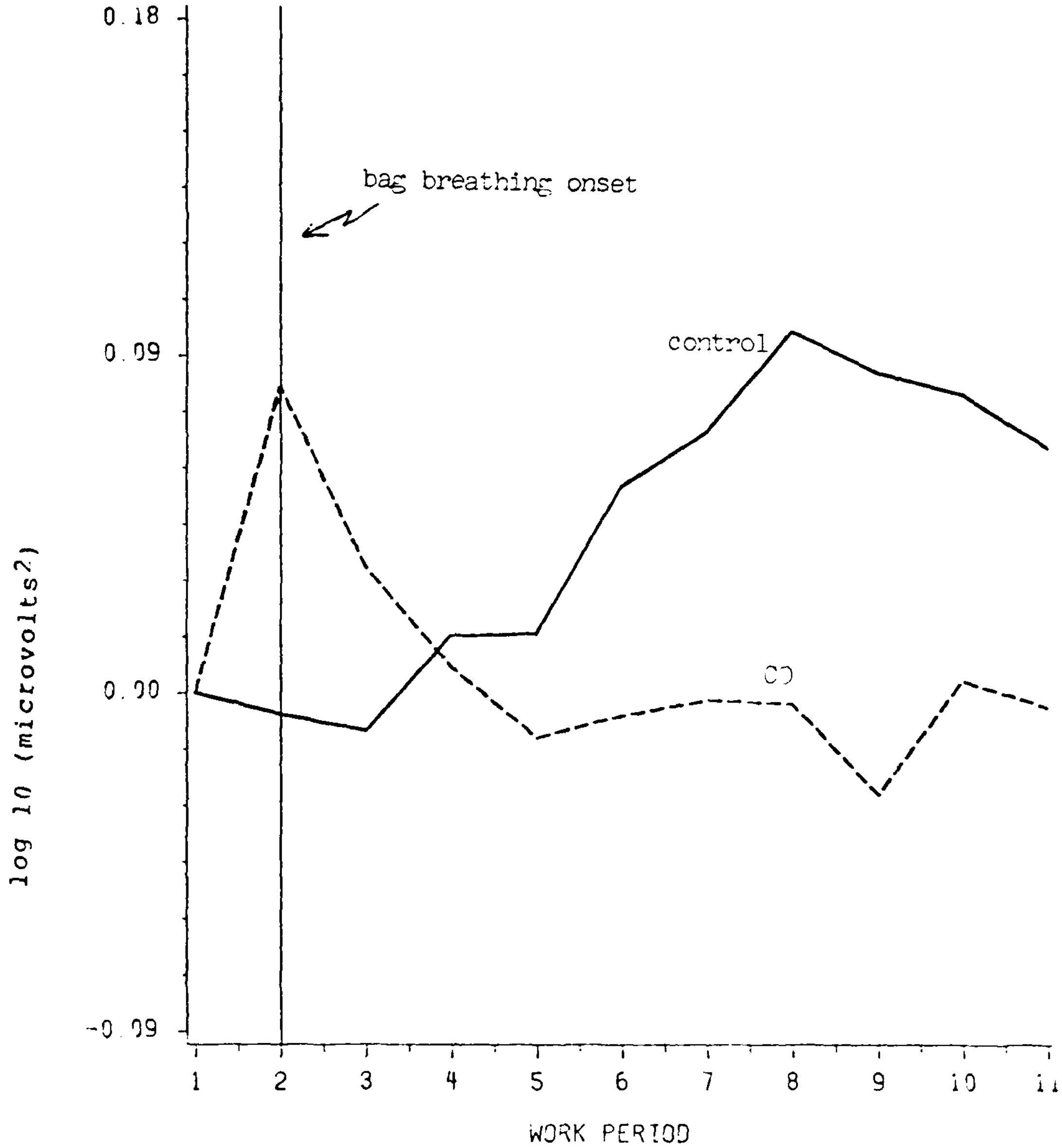

Figure 11. Difference in EEG alpha power from first work period (pre exposure), for work periods 1-10, for CO and control groups.

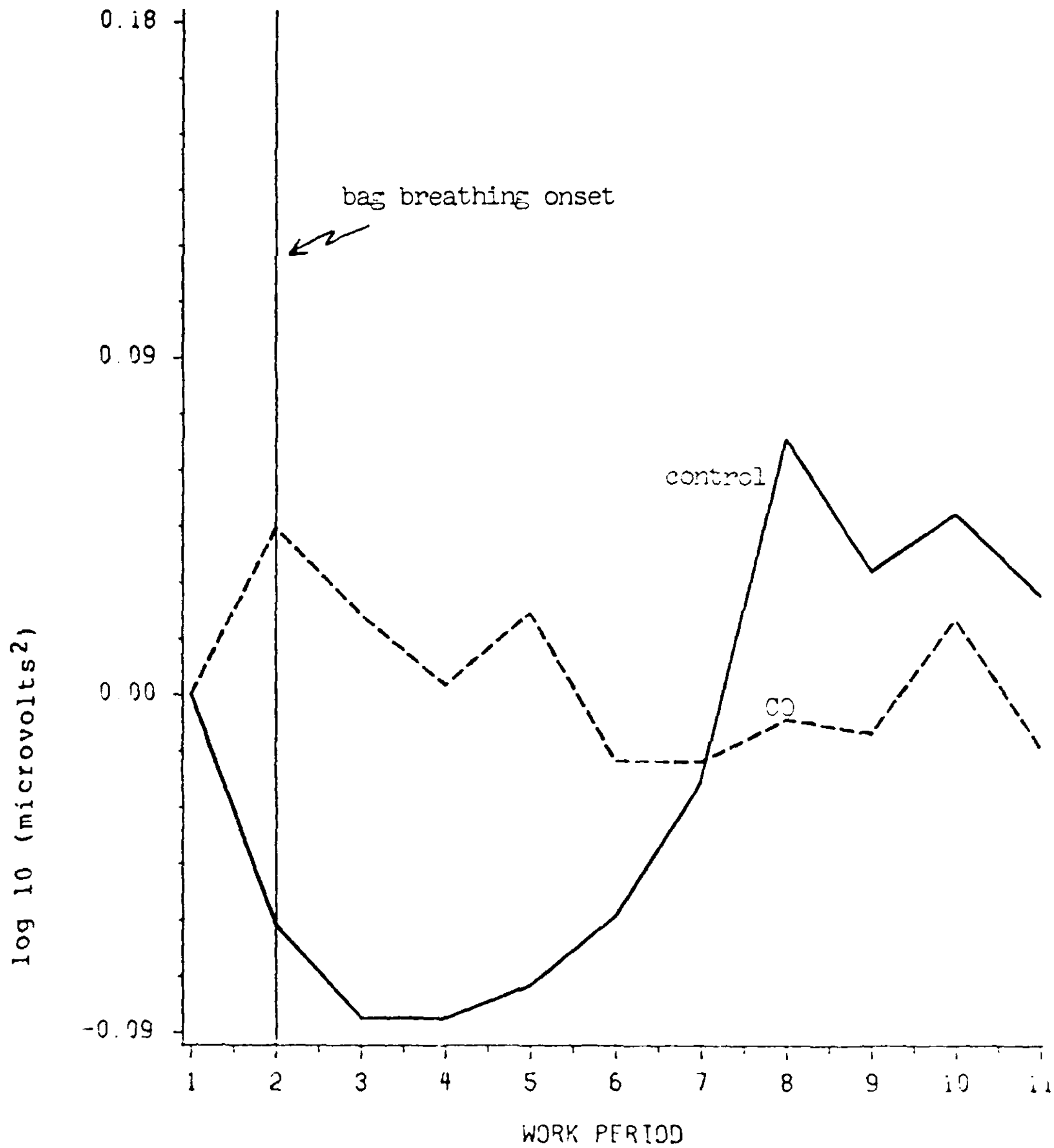

Figure 12. Difference in EEG beta power from first work period (pre exposure), for work periods 1-10, for CO and control groups.

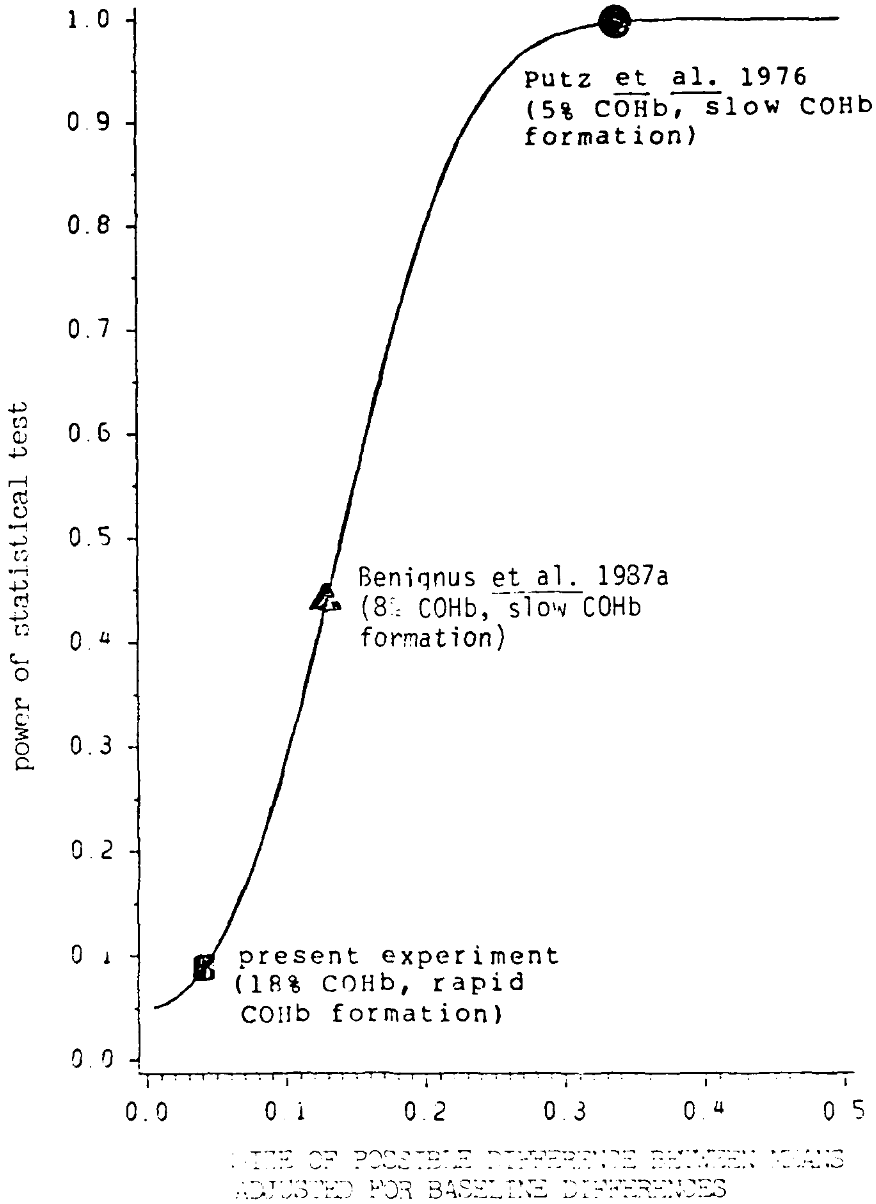

Figure 13. Plot of the power of the test of interaction of pre and post exposure with exposure condition on work period 10. Observed differences in corrected scores in the study by Putz *et al.* (1976), Benignus *et al.* (1987a) and the present study are marked on the curve.

APPENDIX A

RESULTS OF COHb PILOT AND *POST HOC* STUDIES

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RESULTS OF COHb PILOT AND *POST HOC* STUDIES

Introduction

To assure that COHb values could be accurately and safely achieved by use of bag breathing and to assure that the achieved COHb levels could be maintained throughout the experiment, a number of pilot subjects were studied before the main study began. These subjects were not part of the main study and were not counted as part of the sample. The pilot subjects had generally similar characteristics and were given the same screening as discussed above. The protocol was modified as needed in the pilot phase to exploit knowledge gained in the process.

Pilot Study I.

In a simple study of bolus exposure methods, six subjects were exposed to CO calculated to be sufficient to produce a COHb level of either 7, 13, or 19% COHb. Bolus exposure was estimated and achieved in a manner similar to that described in the main study (see METHOD). Each subject was exposed to only one level of COHb. Exposures were conducted in an ascending manner so that any problems would be detected before high levels of COHb were achieved. Subjects had heparin lock catheters inserted into an anticubital vein and blood was drawn on a one minute schedule beginning just before the start of bag breathing and continuing until shortly after the end of bag breathing. After that samples were taken at longer intervals.

Figure A.1 is a graph of the COHb value of the six pilot subjects. Table A.1 is a list of the exposure parameters and

achieved peak COHb. It appeared from these data that the regression method was accurate in predicting peak COHb. To be sure, the peaks were reached at differing times and had differing durations, but the results of the pilot exposures were considered at that time to be sufficiently accurate for purposes of the study.

Pilot Study II

After the pilot work on the bag breathing only, another group of subjects was studied. Subjects performed the task, bag breathed and breathed chamber air with maintenance CO levels. COHb levels were measured before and after the pilot experiment (about 130 minutes after bag breathing). Among this group of pilot subjects, five were exposed to CO. The objectives were (a) to assure maintenance of the COHb levels which had been achieved by bag breathing (b) to correct methodology of the task and (c) to further test the safety of the procedure. The target COHb was 19%. In pilot studiy II, subjects' blood was drawn only before and after the experiment as described above for the main study.

It was assumed that the bolus exposure had produced the intended level of COHb and that the value was to be maintained throughout the pilot (and main study) by the low level of CO in the chamber air. To remove the subject from the chamber in order to draw blood, or to have catheterized him during the long period of the study would have disrupted performance.

The mean COHb, measured at the end of the maintenance period, for the five CO exposed subjects was 19.02%. The standard deviation was 2.25 with a range of 15.7 - 21.4. The

method used above seemed, at the time, to be quite accurate for COHb prediction. It appeared from pilot study I that the method of peak COHb prediction after bolus CO exposure was accurate. From pilot study II it appeared that the method of determining the maintenance dose over the experimental time period was accurate.

The group of subjects in the latter pilot experiment as well as the subjects in the main study covered in the body of this report were used to document that the procedure of bolus CO exposure followed by maintenance CO was safe over the experimental time and did not produce symptoms of CO poisoning such as headaches, dizziness and nausea (Benignus et al. 1987b).

Post Hoc Estimation of COHb in the Main Study

An effort was made to estimate the course of the COHb values throughout the main experiment reported in the body of this article. The Coburn Forster Kane equation (CFKE) (Coburn et al., 1965; Muller and Barton, 1987) was used to estimate the COHb values retrospectively from the final value reached in each subject. The alveolar ventilation (required by the CFKE) was estimated as 70% of the minute ventilation.

Figure A.2 is a plot of the estimated time course of COHb for each of the nine CO subjects. From these curves it would appear that the value of COHb was increasing throughout the course of the experiment. Further investigation revealed that the maintenance CO level used in the experiment had been set higher than intended. The value which had been used was 223 ppm and the intended value was 142 ppm. The error was made in a computer program.

If the maintenance CO level was too high, and yet the final COHb values were near target, then the bolus exposure must have produced less COHb than predicted. Yet, the bolus method seemed to have worked accurately in the pilot studies. It is not clear how the above results may be reconciled. The Stewart _et al._ (1973) regression for bolus exposure and the CFKE make contradictory predictions in this context.

Until further data are obtained, it is not clear what the exact course of COHb levels in this experiment might have been. In any event, the COHb at the end of the study was high and documented.

TABLE A.1. EXPOSURE PARAMETERS AND RESULTING COHb LEVELS FOR BOLUS EXPOSURE IN THE PILOT EXPERIMENT

Subject ID	ppm[1] CO	target COHb	peak COHb
A	3134	7%	8.10%
B	2868	7%	7.27%
C	7370	13%	12.30%
D	6140	13%	13.13%
E	9725	19%	17.20%
F	10178	19%	19.03%

1 The ppm value was calculated using the regression equation of Stewart et al., given the subject's minute ventilation and the desired duration of the bolus.

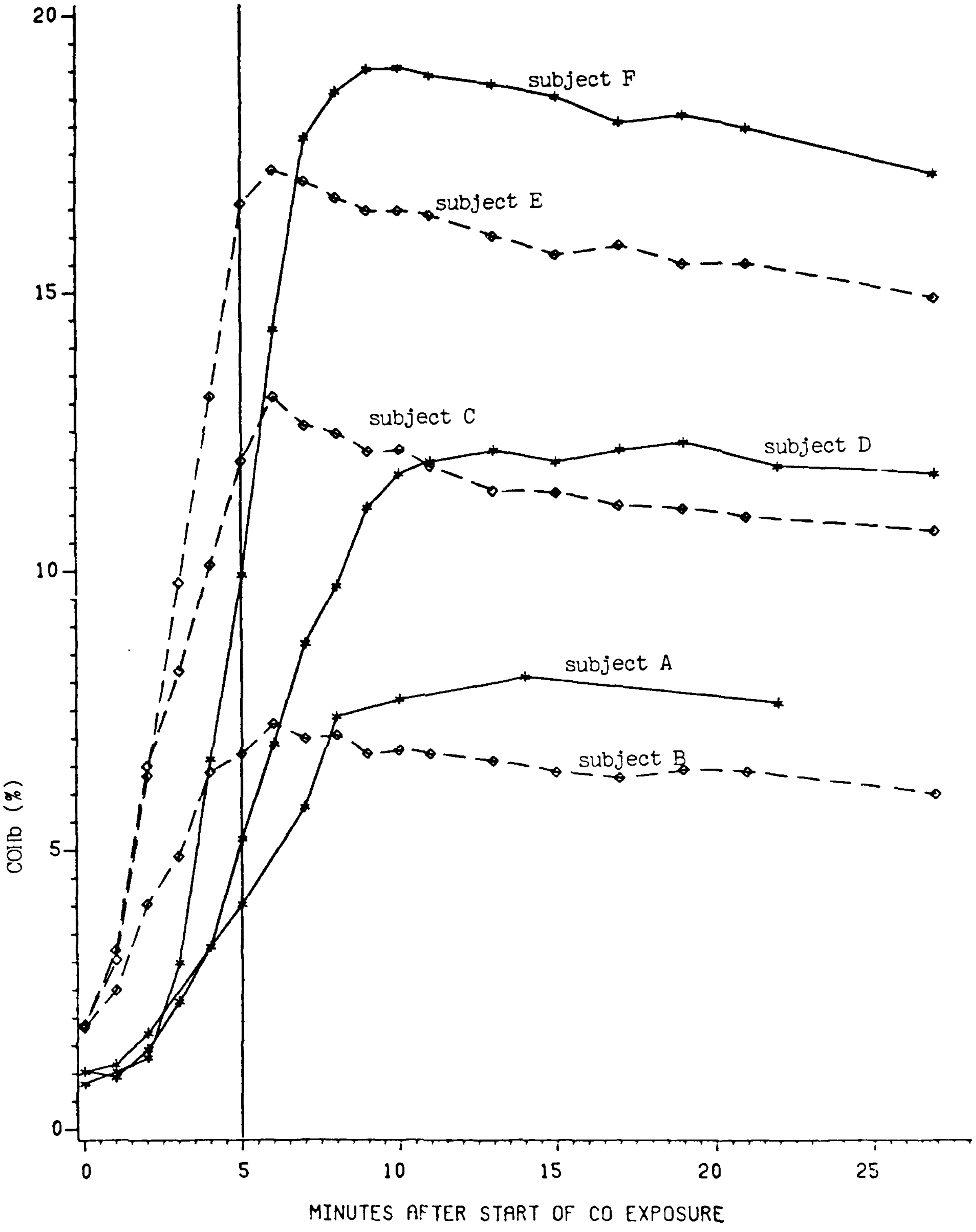

Figure A1.1. Graphs of each pilot subject's COHb value after bolus exposure as described in Table A1.1.

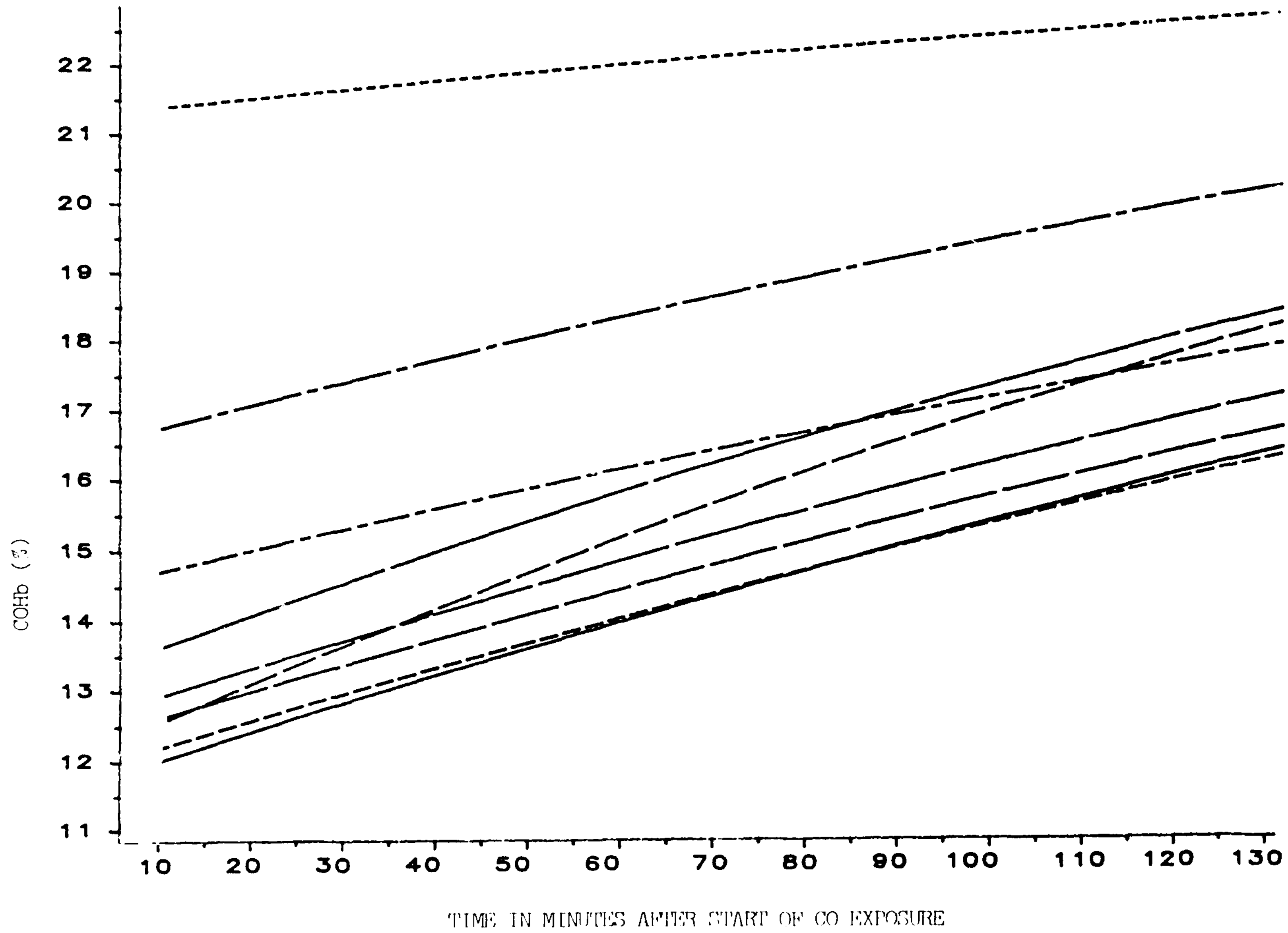

Figure A1.2. Graphs of the estimated COHb for each subject in the main experiment as estimated from the CFKE Using the final value of COHb at the end of the experiment.

APPENDIX B

ANALYSIS OF DISTRIBUTIONAL PROPERTIES OF MAD SCORES

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ANALYSIS OF DISTRIBUTIONAL PROPERTIES OF MAD SCORES

When the tracking data from the Putz *et al.* (1976 and Benignus *et al.* (1987) were converted to the scale of the present study, a problem with the variance became apparent. As seen in Table B.1, the standard deviations varied as a function of the means. Larger means had greater standard deviations. This was especially evident when comparing the means and standard deviations of the present study with those of the other two studies.

Logarithmic transformation of the tracking scores solved the problem. After logarithmic transformation, the standard deviations were no longer dependent on the means and the errors were normally distributed. The independence of the standard deviations from the means is best seen by comparing, in Table B.2, the results from Putz *et al.*, with those of the present study. Although the means were very different, the standard deviations were essentially the same. Standard deviations from Benignus *et al.* were typically higher than those from the other two studies, but collection of standard deviations overlapped with those from Putz *et al.*. More extensive training was used in the present study than in Benignus *et al.* to reduce the variance and increase the power of the statistical test. The effort was apparently successful as can be seen by comparison of the standard deviations from the present study to those of Putz *et al.*

Theoretical justification may be advanced for the use of logarithmic transformations of MAD scores. If the differences

between the FF and the joystick may be assumed to follow a Gaussian ditribution, then variances computed on such differences will be distributed as Chi-square. The logarithm of a Chi-square variable is approximately distributed as a Gaussian variable (Johnson and Kotz,1970). Finally, note that for a Gaussian distribution, the MAD score is a scalar multiple of the standard deviation. Since $\log(S^2) = 2 \log(S)$, the same transformation applies.

TABLE B.1. EARLY AND LATE TRACKING PERFORMANCE FOR THE THREE STUDIES USING MAD SCORES 0-4 SCALE.

	Benignus *et al.* (N=22)				Putz *et al* (N=20)			
	Air		CO		Air		CO	
Hour	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
---slow FF---								
1	.041	.009	.041	.014	.043	.008	.045	.006
4	.035	.007	.043	.014	.041	.009	.049	.006
---Fast FF---								
1	.059	.015	.062	.015	.061	.009	.064	.006
4	.053	.013	.064	.015	.059	.010	.086	.010

Present study (N=19)
Very fast FF

	Air		CO	
Work Period	Mean	± S.D.	Mean	± S.D.
1	1.88	.20	2.03	.26
10	1.73	.21	1.95	.36

TABLE B.2. EARLY AND LATE TRACKING PERFORMANCE FOR THE THREE STUDIES USING ln(MAD) SCORES

	Benignus *et al.* (N=22)				Putz *et al* (N=20)			
	Air		CO		Air		CO	
Hour	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
---slow FF---								
1	-3.21	.212	-3.24	.333	-3.18	.213	-3.12	.137
4	-3.36	.195	-3.19	.322	-3.23	.240	-3.02	.127
---Fast FF---								
1	-2.85	.236	-2.80	.241	-2.81	.144	-2.75	.094
4	-2.96	.247	-2.78	.230	-2.85	.164	-2.46	.133

Present study (N=19)
Very fast FF

	Air		CO	
Work Period	Mean	± S.D.	Mean	± S.D.
1	.625	.110	.699	.131
10	.543	.120	.651	.179

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